EFFECTS OF DIETARY WHOLE COTTONSEED ON LACTATIONAL PERFORMANCE AND DIGESTIBILITY AS RELATED TO ROUGHAGE SOURCE, PROTEIN SOURCE AND CALCIUM TREATMENT

BY

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1987

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. D.K. Beede, chairman of his supervisory committee, for the guidance provided him throughout this study and for the invaluable suggestions towards the preparation of this manuscript. Sincere appreciation is extended to Drs. B. Harris, Jr., C.J. Wilcox, H.H. Van Horn and O.C. Ruelke, members of his supervisory committee, for their support, encouragement and understanding.

Thanks also are extended to fellow graduate students for their readiness to share with him their knowledge and experience. He is grateful to the following individuals for their involvement in his research: Anita O'Connor, Buhri Ariffin, Chang-Zheng Wang, Darwinsyah Lubis, Dale Hissem, Humberto Ruiloba, Lokenga Badinga, Mark McGuire, Masri Muhamad and Stephen Emmanuel; to Drs. C.R. Staples and K.C. Bachman for their invaluable suggestions and comments.

Appreciation is expressed to Estelle Hirchert for technical assistance with sampling and analyses of samples, and to Angelita, Cheryl and Joyce for their assistance in the laboratory. The assistance given to him by the entire farm crew under the leadership of Austin Greene towards his experiments at the Dairy Research Unit is gratefully acknowledged.

He is most grateful to the Malaysian Agricultural Research and Development Institute and the Government of Malaysia for granting him scholarship and study leave, and for their confidence and kind consideration shown towards him in allowing for extensions of his study leave.

Most of all he has warmest gratitude to his wife, Azizah, and children, Azlina, Bakhtiar and Adzhar. No word can adequately express his appreciation for their patience, tolerance and understanding throughout the duration of study.

Finally, the author acknowledges partial grant support from Cotton Inc., Raleigh, NC, and thanks Cindy Zimmerman for typing this manuscript.

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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May 1987

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Major Department: Dairy Science

Feeding of whole cottonseed (WCS) in diets for dairy cows was studied. In Experiment I, 36 mid-lactation Holstein cows were assigned randomly in a partially balanced incomplete block design with a 3x3x2 arrangement of treatments. Dietary factors were WCS = 0, 15 and 30% of dry matter (DM), roughage sources (CS = corn silage, AH = alfalfa haylage and CSH = cottonseed hulls) and dietary calcium (.69 and 1.29% of diet DM). Increasing WCS decreased DM intake, milk fat (MF) percent and milk protein (MP) percent. Feed DM intake was higher than with CSH- than AH- or CSH-based diets. Actual and fat-corrected milk (FCM) yields were similar for all three roughage-based diets. Significant WCS x roughage interactions existed for FCM yield and MF percent. Inclusion of WCS at 30% of diet proved detrimental to FCM yield, MF percent and MP percent.

In Experiment II, eight nonlactating Holstein cows were used in two digestion trials and fed diets containing 20% WCS with or without calcium hydroxide treatment to initiate soaping. Calcium was equalized among diets using limestone. Designs were partially balanced incomplete blocks with 2x2x3 arrangement of treatments. Each period was 28 d. Calcium hydroxide treatment reduced DM intake, lowered acetate:propionate, and lowered coefficients of apparent digestibility of DM and acid detergent fiber (ADF). Ruminal ammonia and total volatile fatty acids concentrations were higher with treated WCS than with untreated. Sources of supplemental proteins did not affect digestion coefficients, but soybean meal (SBM) resulted in higher ruminal estimates than distiller's dried grains plus solubles (DDGS).

The final experiment involved in situ disappearance of CSH and grass hay DM and ADF. Objectives were to characterize disappearances (rate and 72-h extent) of these roughages and 72-h extent of WCS disappearance, and estimate effects of dietary factors as in Experiment II. Extents of 72-h DM disappearance were 58.3 and 42.3% for CSH and hay, but only 28.8% of the WCS DM disappeared. Calcium hydroxide treatment of WCS depressed disappearances of DM and ADF as reflected by longer lag times and slower rates of disappearances. Supplemental SBM caused a faster and more extensive disappearance than DDGS.

CHAPTER I
INTRODUCTION

One of the greatest attributes of ruminants is their unique ability to utilize fibrous materials for food. In many parts of the world, poor quality roughages such as cereal straws, sugarcane bagasse and other crop residues form the major portion if not the sole diet of the ruminants. These feedstuffs occur in abundance. However, by themselves, such roughages cannot supply enough nutrients to support a reasonable level of productivity.

Concentrate supplementation can help improve utilization of low quality roughages and by-products. Cereal grains which form the base ingredient in concentrate supplements have nearly twice the energy of average quality roughages, and they contribute less to heat increment than roughages (NRC, 1982). Supplementation is inevitable for high producing cows whose requirements are not easily satisfied from roughages alone. In the United States, the feeding of cereal grains to ruminants is common practice, accounting for over 40% of the 150 to 200 million metric tons used for livestock annually (Elliott and Bauman, 1980).

There is a growing interest in feeding oil seeds, such as whole cottonseed, to early-lactation and/or high producing cows because of its unique balance of high energy (fat), high protein and also high effective fiber. The last component is important in maintaining fat percent in milk. There are characteristics inherent in whole cottonseed which might limit the maximum amount that can be included effectively and safely in the diet. Its high oil content may affect adversely fiber digestion in the rumen if included in too large amounts. A possible method for alleviation of this problem is through soaping the fatty acids with dietary calcium. This has been demonstrated successfully in the laboratory (Jenkins and Palmquist, 1982) but whether it can be a practical tool for direct farm application with whole cottonseed is still a question to be answered. Some calcium sources are more effective than others.

There are results (Smith et al., 1981) which suggested that some of the fats in whole cottonseed actually may escape digestion in the rumen. It would appear beneficial, therefore, to feed whole cottonseed with a source of protein that has low ruminal degradability. Low milk protein percent is a common problem with feeding of whole cottonseed. Inconsistency in milk fat percent response to whole cottonseed feeding has led to suggestion of possible roughage by whole cottonseed interaction.

Research reported herein on incorporating whole cottonseed in dairy rations was conducted with these objectives:

1. To study effects of dietary levels of whole cottonseed, limestone and sources of roughage on feed intake, milk yield and composition, and apparent digestibility of dietary dry matter and fiber components.

2. To compare supplementation of distiller's dried grains plus solubles and soybean meal as potential complementary protein sources in whole cottonseed-containing rations on ruminal digestion.

3. To determine effectiveness of calcium hydroxide treatment of intact and crushed whole cottonseed on ruminal soaping, apparent digestibility and in situ disappearance of dry matter and acid detergent fiber from the rumen.

CHAPTER II
REVIEW OF LITERATURE

Traditionally, roughages form the bulk of ruminant diets. These include grasses, legumes, silages, straws and other by-products high in fiber. Such feedstuffs are relatively low in available energy and often are supplemented with concentrates (feedstuffs containing less than 18% crude fiber) which are higher in nutrients, particularly crude protein and in energy to meet high requirements for production. High starch-containing cereal concentrates are used frequently to supply additional energy.

In spite of this, many high producing dairy cows are unable to consume enough feed to meet their high energy and nutrient demands during early and peak lactation, and frequently go into negative energy balance because of physical limitation imposed by rumen fill and rate of passage of feedstuffs in the digestive tract (Freer and Campling, 1963). These animals must draw upon their energy reserves in adipose tissues for such needs. Alternatively, energy concentration of the diet can be increased through supplementation with starch concentrates or fat. Oftentimes, high starch concentrates can lead to subclinical ruminal lactic acidosis which depresses productivity (Dirksen, 1970). Therefore, an alternative to higher starch feeding may be to feed more dietary fat. This subject has been reviewed by Palmquist and Jenkins (1980).

Fat contains 2.25 times as much energy as carbohydrate on a weight basis. Because of this higher concentration of energy, its inclusion in the diet will enable replacement of a significant portion of grain in a high concentrate diet. High grain diets lower the pH of the ruminal fluid making it less favorable for fiber digestion. However, there is no evidence of effects on ruminal pH with high dietary fat.

Ordinarily, fats comprise less than five percent of ruminant diets, with up to 50 and 20% of total ether extract being nonfatty acid materials such as pectin and waxes in forages and grains, respectively (Palmquist and Jenkins, 1980).

Effects of Dietary Lipids on Rumen Metabolism and Animal Performance

Metabolism of Dietary Lipids in the Rumen

Fats in feedstuffs exist chiefly as glycerol esters of fatty acids, or so-called triglycerides (TG). Upon entering the rumen TG are hydrolyzed rapidly to yield high concentrations of unesterified fatty acids by the action of extracellular lipases in the ruminal fluid (Noble, 1978), which may have their origin in the plant tissues. Where unsaturated fatty acids are present, specifically linoleic (C18:2) and linolenic acids (C18:3), biohydrogenation occurs almost immediately upon hydrolysis of the TG (Harfoot, 1978). However, it has been shown that hydrolysis is a prerequisite to ruminal biohydrogenation (Hawke and Silcock, 1969). Although the former is a rapid and complete process, there are indications that it may be the rate-limiting step in biohydrogenation.

As the final outcome of these processes, unesterified fatty acids become the predominant lipid fraction of the digesta in the rumen. These fatty acids are associated physically with the particulate matter in the rumen and are adsorbed largely onto the particles (Noble, 1978). By far the major portion (80%) of the total fatty acids in the digesta at this stage is stearic acid (C18:0) (Palmquist and Jenkins, 1980). Small quantities of oleic (occurring predominantly as trans-11 isomer), linoleic and linolenic acids are present also. Together, these unesterified long chain fatty acids constitute the major dietary lipid supply to the host animal, while about 20% of the total lipid supply comes from the protozoal and bacterial contribution.

End-products of ruminal fermentation of dietary carbohydrates are chiefly acetic, propionic and butyric acids. These short chain fatty acids (volatile fatty acids) occur in varying proportions depending on nature of the diet. These fatty acids are absorbed through the rumen wall and go into the portal circulation supplying the host animal with between 60 and 80% of the total digestible energy (Harfoot, 1978). About 70% of total production of ruminal short chain fatty acids is absorbed within the rumen itself. However, with the longer chain fatty acids, only a negligible amount is absorbed through the wall of the rumen into the lymphatic system relative to the total amount present in the ruminal digesta (Noble, 1978).

Effects of Dietary Lipids on Animal Performance

Effects of fat supplementation in the diet vary greatly depending upon particular situations under which the studies were conducted. Generally energy intake is increased (Smith et al., 1978) without depressing the ruminal pH as compared with high grain feeding. Dry matter intake, however, may show a decrease for cows consuming fat-suppplemented diets (Smith et al., 1978; Palmquist and Conrad, 1980; Clapperton and Steele, 1983), but the high energy density of fat will result in a higher total energy intake compared with diets containing no supplemental fat (Palmquist and Jenkins, 1980). This increased energy intake often leads to increased production of fat-corrected milk.

Trends toward a decreased milk protein percent and production are evident from results of Dunkley et al. (1977), Emery (1978), Palmquist and Conrad (1980), Hawkins et al. (1982). Other studies, however, showed no change in milk protein percent (McGuffey and Schingoethe, 1982; Rafalowski and Park, 1982). Types of fat used in these studies varied and may explain variable responses.

One other effect associated with high fat feeding concerns the digestibility of fiber. Erwin et al. (1956) reported reduced digestibility of dry matter and crude fiber in steers fed a high fiber diet when supplemented with 7% animal fat. Reduced fiber digestibility with fat supplementation of a low quality roughage diet was noted by Ward et al. (1957). High fat supplementation was implicated to reduce fiber digestion (Palmquist and Jenkins, 1980; Jenkins and Palmquist, 1982; McAllan et al., 1983). This negative effect on fiber digestion is most likely thought to be due to direct inhibition of microbial growth and activity (Harfoot, 1978) from surface-active effects of fatty acids on bacterial cell membranes. As a possible consequence of the reduced fiber digestion, proportion of acetate to propionate may be reduced. It has been suggested that a diminished availability of acetate would lead to a reduction in fatty acid synthesis in the mammary gland (Christie, 1978). The degree of depression in fiber digestion is greater with free and unsaturated fatty acids compared with saturated fatty acids (Clapperton and Steele, 1983).

Palmquist and Conrad (1978) suggested that minimal fiber digestion inhibition is likely when fat is supplemented in a high forage diet. An early experiment from Georgia (Smith et al., 1916) reported the use of cottonseed oil in dairy rations. In this study it was found that addition of cottonseed oil to the ration produced changes in the properties and composition of butterfat. These workers suggested that the dietary fat was first altered in the rumen rather than being directly absorbed and transferred to the milk. Anderson et al. (1979) observed higher proportions of C18 fatty acids from cows receiving whole cottonseed (WCS) than from control cows.

Moody (1978) experimented with the feeding of cottonseed oil in the form of either WCS (replacing 19 and 16% of the concentrate portion) or cottonseed meal plus the equivalent amount of the crude extract oil. He found no differences due to the mode of oil feeding and noted that either method resulted in depressing effect on quantity of milk fat produced.

Brown et al. (1962) studied the effects of tallow supplementation to diets when roughages were fed at 1.0 and 2.5% of body weight. The results showed a small but significant increase in milk fat percent with the higher roughage diet. In another study (Wren et al., 1978), there was an increase in milk yield with a small decrease in milk fat percent with fat supplementation in the diet. The total milk yield, however, was not different. The level of supplementation with tallow was equal to 18% of the total digestible energy of the diet. Feed intake was not affected adversely.

Studies of Macleod et al. (1972) and Macleod and Wood (1972) on tallow supplementation showed no significant effects on milk yield or milk fat composition. Hatch et al. (1972) added tallow as supplemented fat at levels of 0, 3 or 6% of the diet to feedlot steers. There was a 7 and 5% reduction in the quantity of acetate and butyrate, respectively, with the addition of 3% fat to the diet. The diet with 6% added fat caused an overall reduction in nitrogen retention.

Johnson and McClure (1973) experimented with adding partially-hydrolyzed animal and vegetable fat (HEF) or corn oil to chopped corn prior to ensiling. They reported that addition of fat did not alter the fermentation process in the silo. There were significant reductions in the digestibility of organic matter, dry matter and cellulose at 4% HEF supplementation but not at 8 or 12%. Digestibilities were lower with 8 than 4% corn oil addition. Considerable amounts of fecal soaps were formed from rations containing the HEF and the amount formed increased with increasing level of fat in the silage. There was no apparent overall effect of limestone on digestibilities of HEF silages, but it alleviated digestibility depression of silages containing 8% corn oil.

Murphy (1982) reported a series of experiments involving tallow supplementation to the concentrate portion of a ration for cows fed grass silage. Addition of tallow at 20 g/kg concentrate mixture (based on beet pulp) caused a 15% increase in silage intake, but when tallow was included at 40 g/kg concentrate, silage intake dropped slightly. A related experiment (Murphy, 1982), using good quality silage, showed a 10% increase in silage intake when the concentrate was supplemented with 67.5 g tallow/kg concentrate. When the concentrate was fed in isocaloric amount to a concentrate with no fat, there was still a 14% higher silage intake by cows receiving the fat supplemented concentrate.

Van der Honing et al. (1981) conducted balance trials using dairy cows and wethers to study effects of fat supplementation of concentrate on digestion and utilization of energy. They found that supplementation with tallow (5 to 7%) or soybean oil (5%) to a concentrate mixture did not cause any significant depression of energy or fiber digestibility. Fat supplementation in the form of soybean oil depressed the milk fat percent and therefore lowered the milk energy yield.

In another study, de Visser et al. (1982) evaluated fat-supplemented concentrates fed to lactating cows. Results indicated an increase in milk yield, but a decrease in milk fat percent overall, and also a lower milk protein percent with 12% supplemental fat to the concentrate fraction. Supplementation of diets with unsaturated oils such as soybean oil and cottonseed oil have shown a tendency towards a reduced milk fat percent (Brown et al., 1962; Macleod and Wood, 1972; Astrup et al., 1976; Moody, 1978).

Depression in percent milk fat may occur under conditions leading to a lower acetate to propionate ratio (Davis and Brown, 1970) such as when fiber digestibility is reduced by high polyunsaturated fat supplementation in the diet. According to Palmquist (1976), such changes in acetate to propionate ratio would induce the adipose tissue to compete for acetate thereby reducing its availability to the mammary gland. This would be accompanied by increased uptake of long chain fatty acids by the adipose tissue and a reduced mobilization of adipose long chain fatty acids for synthesis of milk fat (Davis and Brown, 1970).

Protection of Fat Against Ruminal Degradation and Protection of Ruminal Fermentation Against Dietary Fat

The preference for high polyunsaturated over saturated fat in the human diet has influenced the direction of ruminant nutrition research somewhat (Clapperton and Steele, 1983). The last decade saw trends toward producing fats that are lower in saturation and in trans acid content through the feeding of protected fat. Ruminant fats typically are highly saturated and contain very low proportions of polyunsaturated fatty acids despite the fact that they are traditionally rich in polyunsaturated fatty acids (Hilditch and Williams, 1964). Linoleic and linolenic acids together constitute almost 75% of the total fatty acid content of grasses (Hilditch and Williams, 1964), and 67, 55 and 60% in clover pasture, alfalfa hay and soybean seeds, respectively (Palmquist and Jenkins, 1980; Van Soest, 1983). However, milk and meat contain only 4% linoleic plus linolenic acids (Hilditch and Williams, 1964).

The apparent lack of transfer of polyunsaturated fatty acids in the diet to animal products, under normal circumstances, is due to biohydrogenation by certain ruminal microbial strains (Hawke and Silcock, 1969). This was confirmed by studies such as those of Tove and Mochrie (1963) who reported substantial increases in linoleic acid in milk fat when cottonseed oil emulsion was injected into the jugular vein, thus avoiding ruminal microbial biohydrogenation.

Coating the supplemental fat or protecting it in some form may help alleviate some of the problems associated with fat supplementation (Mattos and Palmquist, 1974; Bines et al., 1978; Jenkins and Palmquist, 1981, 1982). Such manipulation will prevent the fat from interfering with the ruminal fermentation process which results in higher milk fat (Astrup et al., 1976; Dunkley et al., 1977; Bines et al., 1978; Smith et al., 1978; Kronfeld et al., 1980).

Traditionally, protection against ruminal microbial degradation has been through chemical treatment involving formaldehyde-protein capsules as described by Scott et al. (1971). Such a method would be expensive. A possible alternative to formaldehyde-protein capsules could be formation of fatty acid soaps of calcium (Palmquist and Conrad, 1980; Jenkins and Palmquist, 1982). Earlier, Brooks et al. (1954) reported that addition of alfalfa ash (high in calcium) helped alleviate the problem of reduced ration fiber digestibility associated with fat feeding. Thus, calcium soaping of dietary fatty acids has been given consideration lately as a means of supplying ruminally-protected fatty acids in the diet of the ruminant.

A technique developed in Australia (Scott et al., 1971) was shown effective in alleviating the problem of ruminal biohydrogenation of polyunsaturated fatty acids by the microbes. Basically, it involved coating tiny fat droplets in a formaldehyde-protein coat. The resulting complex is resistant to degradation under normal conditions in the rumen and therefore protects the fatty acid against microbial hydrogenation. Under the acidic conditions of the abomasum (pH 2 to 3), the complex dissociates releasing the fat droplet for subsequent absorption and transfer to plasma, milk and depot fats.

Changes in fatty acid composition in beef and milk can be effected through dietary manipulation. Concentrations of polyunsaturated fatty acids in these products can be increased by feeding fats that are protected against ruminal biohydrogenation (Bauman and Davis, 1974). Numerous reports (Cook et al., 1972;

Garrett et al., 1976; Mills et al., 1979; Clapperton, 1982) have indicated success in increasing linoleic acid content of fats in ruminants. Garrett et al. (1976) reported increased linoleic content of depot fat of cattle from 5.3 to 15.7% when formaldehyde-treated sunflower seeds were incorporated into a fattening diet. Likewise, through supplementation with protected sunflower oil (formaldehyde-casein coated), it was possible to increase linoleic acid content to 25% of the depot fat in lambs (Mills et al., 1979) and to over 30% in milk fat (Cook et al., 1972).

Clapperton (1982) reported significant increases in linoleic acid content in milk when a low roughage diet was supplemented with sunflower oil coated with formaldehyde-casein. Comparable responses were found with safflower oil similarly protected against ruminal biohydrogenation (Bitman et al., 1973). They reported transfer of between 17 to 42% of the dietary linoleic acid to milk fat. Levels of intake of the linoleic acid ranged between 105 and 1196 g/d. In many cases these responses were accompanied by actual increases in milk fat percent.

Various studies, reviewed by Palmquist and Jenkins (1980), demonstrated the beneficial effects of cation addition to the diet in reversing the inhibitory effects of fat on fiber digestion. Effects have been attributed mainly to calcium in the form of ash (Brooks et al., 1954) and dicalcium phosphate and calcium chloride (Jenkins and Palmquist, 1982). Calcium ions combined with fatty acids in solution thereby forming insoluble soaps rendering the fatty acids unavailable to bind to ruminal microbial cells. Work of Palmquist and Conrad

(1980) and Smith et al. (1981) provide further evidence of the possible effects of calcium in countering negative effects of fatty acid on fiber digestion.

Jenkins and Palmquist (1982) studied the effects on plant cell wall digestibility in vitro, by the addition of 10% tallow to a diet consisting of 70:30, roughage to concentrate, with and without supplemental calcium. They observed a markedly lower cell wall digestibility of the diet with added fat. In the presence of additional calcium in the form of dicalcium phosphate or calcium chloride, there was increased formation of insoluble fatty acid soaps, and consequently an improvement of cell wall digestibility over the treatment not containing additional calcium. Calcium chloride was more reactive than dicalcium phosphate in complexing with the nonesterified fatty acids and this took effect as early as 6 h after incubation. Supplied as calcium carbonate in the diet, calcium was not effective in forming fatty acid soaps in the rumen (Drackley et al., 1985).

Cotton By-Products in Animal Feeding

Cotton by-products that have been used in rations for livestock include cottonseed meal (CSM), cottonseed hulls (CSH), cottonseed oil and WCS. It is estimated that for every ton of lint produced from seed cotton, there is associated with it 1.7 tons of cottonseed (Gohl, 1981). The seed upon extraction would yield approximately 20% by weight in edible oil and residue, comprising 50 and 30%, respectively, of CSM and CSH. Availability of cottonseed meal for use as concentrate feeds in the United States was estimated at 1.9 million tons/year over the past decade (Jones, 1981). Cottonseed meal provides a source of protein and energy in rations for poultry, swine, and beef and dairy cattle. Cottonseed hulls provide a source of fiber. Its form renders it most suitable for use in complete rations (McCoy et al., 1965). Recent developments in dairy operations dictated increased use of WCS for feeding to lactating cows (Amos, 1984). Its increasing popularity as a feed ingredient is attributed partly to its relatively high fiber content, while at the same time providing a concentrated source of energy in the fat fraction.

Cottonseed Meal

Cottonseed meal, a by-product remaining after oil extraction of whole seed, has found wide use as a supplemental source of protein particularly for poultry and swine. The different methods of extraction of seed oil viz. the screw press, prepress solvent extraction and direct solvent extraction produce meals of different qualities particularly in relation to levels of residual lipids, free gossypol and quality of protein (Jones, 1981). Cottonseed meal extruded from the screw press characteristically is lower in protein quality and in free gossypol. Extraction of oil by use of solvents produced a meal that is moderate to high in protein quality, but the free gossypol content also tends to be higher. On the average, crude protein content of cottonseed meal is 41 to 42% (Jones, 1981) and ranges between 40 and 54% depending on method of processing (NRC, 1978).

For ruminants, the value of cottonseed meal proteins, like other proteins, is influenced by the proportion of amino acids that escape microbial digestion in the rumen. Cottonseed meals vary considerably in ruminal degradability, resulting in different bypass characteristics (Jones, 1981). Processing temperature of cottonseed meal is an important factor in influencing the extent of protein binding which determines its degradability in the rumen (Jones, 1981). Meals extruded from a screw press usually have a low rate of ruminal solubility and, therefore, may be expected to be better utilized by the animal if heat damage does not occur. A higher proportion of the amino acids would be available in the hindgut for digestion and assimilation by the animal.

Zinn et al. (1981) compared ruminal degradability of different supplemental proteins in steer calves fitted with duodenal cannulas. Supplemental proteins were casein, soybean meal, cottonseed meal, corn gluten meal, linseed meal, and meat and bone meal which were used to provide 27% of the total N of the diet based on 40% alfalfa hay and 60% concentrate. Their results indicated that, when fed at 3 kg/d of the supplement, these diets ranked as follows in the order of resistance to ruminal microbial degradation: corn gluten meal greater than cottonseed meal greater than soybean meal greater than casein. Fed at 4 kg/d, cottonseed meal protein ranked equal to corn gluten meal but was inferior to meat and bone meal in resistance to microbial degradation. There was no mention, however, about whether the different types of meals were obtained by a common process. The authors suggested possible associative effects of feed ingredients used in the experiment. Nevertheless, the results clearly indicate that cottonseed meal fares better than soybean meal in escaping ruminal microbial degradation.

Van Horn et al. (1979) conducted a study in which comparisons were made between milk yield responses to cottonseed meal, soybean meal and peanut meal. Results indicated that, with 13.5% meal supplementation, milk yield/kg feed intake was higher on a ration supplemented with cottonseed meal than either soybean meal or peanut meal, but not at 16.3%. Total tract digestibility of dietary organic matter was lower for the cottonseed meal-supplemented diet than either soybean meal or peanut meal at either level of supplementation.

Cottonseed Hulls

Cottonseed hulls are useful as an alternative fiber source to forage, silage or hay, particularly in rations where forages are lacking. Its total digestible nutrient value is around 38% (NRC, 1978), and its fiber is relatively high in digestibility. Though nutritionally it is not comparable generally to forage or hay, it can be used readily as an ingredient in complete feeds because of its desirable physical form. In addition, it can be packed, handled and stored without much inconvenience.

Inclusion of CSH in rations has been effective as a fiber source for cattle. Dry matter intake is consistently higher in cattle fed CSH rations compared with other sources of roughage including alfalfa hay or haylage, corn silage or corn cob (Benz et al., 1966; Hale et al., 1969; Villavicencio et al., 1968). Vernlund (1979) reported a reduced intake of dry matter when CSH were fed in pelleted form compared with regular CSH. When pelleted, CSH rations produced less milk and a lower milk fat percent compared with loose CSH. Feed intake was lower also.

Milk yield responses to rations containing CSH are variable. Villavicencio et al. (1968) reported no significant difference in milk yield of cows fed a ration containing CSH compared with a control ration based on corn silage. Similarly, Brown et al. (1977) reported no difference in milk yield when cows received CSH in substitution for up to 50% alfalfa hay cubes, whereas McCoy (1965), and Sargent et al. (1975) reported increases in milk production. There was no difference in 4% fat-corrected milk yield between a 40% CSH-containing ration and a 30% alfalfa haylage-containing ration (Benz et al., 1966). Furthermore, milk yield with either ration was greater than a ration with 30% CSH. Similar results were reported by Olson and McCoy (1975), comparing 30 and 40% CSH-containing rations. No differences in milk composition were reported.

Sargent et al. (1975) reported a reduced digestibility of a ration containing 30% CSH compared with one containing 50% corn silage. Percent oragnic matter and dry matter digestibilities of rations containing 30% CSH were 48 and 47%, respectively (Vernlund, 1979).

Value of Whole Cottonseed for Lactating Cows

Feeding WCS offers certain advantages to ruminants. It has been a promising alternative especially for use in supplementing diets of cows in early lactation (Anderson et al., 1979; Smith, 1983) and in situations where intake was depressed because of heat stress (Moody, 1962). It is high in both energy and protein (each about 23%) and fiber which is readily digestible. Much remains to be learned regarding its pattern of digestion and metabolism in the rumen and the various factors affecting it.

Replacement value of WCS was studied by Davis and Harland (1946) who replaced 1.36 kg cottonseed meal and 1.82 kg corn with 3.18 kg WCS. In this case, replacement was not very accurate because of the higher energy concentration of the oil in WCS. Moreover, WCS was relatively higher in fiber content. They reported average milk fat percent increases were .13% and .24% from two different groups of cows compared with control diets containing no WCS. Cows were allowed free access to alfalfa hay.

Anderson et al. (1979) used 1.91 kg WCS in a 1:1 substitution for cereal grain concentrate in the diet of 20 Holstein cows. They observed an increase in milk yield with WCS feeding, but feed dry matter consumption was similar for both diets. There was no effect on milk fat percent. However, milk protein percent was lower with the WCS diet. These workers further demonstrated in a related trial that even when the diets were made isoenergetic, there was still a superior response from the WCS diet in terms of milk and solids-not-fat production. Other responses were not different.

DePeters et al. (1985) experimented with four levels (0, 10, 15 and 20% of total ration, dry basis) of WCS feeding to Holstein cows of various ages and stages of lactation. They similarly reported decreased milk protein percent with WCS feeding. Milk yield and milk fat percent were improved with WCS substitution for concentrate in a chopped alfalfa hay-based diet.

In a series of experiments (Van Horn et al., 1984) evaluated effects of WCS in isofibrous diets using hulls from cottonseed, sunflower, peanut, sugarcane bagasse and corrugated boxes as sources of roughage. They reported depressed milk fat percent but higher feed intake and milk yield from WCS supplementation. More interestingly, there was a WCS by roughage source interaction effect on feed intake and milk yield. Compared with diets that contain no WCS, inclusion of the seed in a steam-pressure treated, bagasse-based diet stimulated a bigger increase in dry matter intake than when supplemented in a corn silage diet. Diet dry matter intake of CSH-based diet was depressed when containing WCS.

Bernard and Amos (1985) reported that pelleting of WCS tended to stimulate the cows to eat more dry matter and produce more milk than when the seeds were fed loose. There were no differences in any of the responses studied except the total solids and lactose contents of the milk which were improved with pelleted WCS. In a complementary digestion trial involving Holstein steers fitted with ruminal and abomasal cannulae, a higher crude protein content associated with the particulate digesta was found in the abomasum of animals receiving the pelleted cottonseed than WCS. This indicated a possible lower degradation of protein in the pelleted cottonseed consequent to the pelleting process.

It has been demonstrated, from in situ data (Pena et al., 1982), that WCS dry matter disappearance from the rumen dropped linearly when seeds were subjected to autoclaving temperatures from 120 C to 180 C for 20 min. Crude protein disappearances (%) were 92.7, 86.8, 74.7 and 48.2 with heating temperatures of 0, 140, 160 and 180 C, respectively.

Many other studies also reported depressions in milk protein and solids-not-fat percents associated with increased milk fat from the feeding of WCS (Smith et al., 1981; Dale et al., 1984; dePeters et al., 1985) and from addition of protected tallow to the concentrate ration (Dunkley et al., 1977; Macleod et al., 1972). Only two experiments have demonstrated reduced milk fat percent (dePeters et al., 1985; Van Horn et al., 1984).

High fat content of WCS (in excess of 20% of dry matter) likely would impose a limit on its inclusion in rations. for ruminants, particularly for lactating dairy cows because of possible negative effects of fat on ruminal fiber digestion and milk fat content as reviewed earlier. Smith et al. (1981) reported no negative effects of fat in WCS on fiber digestibility. It was further reported that inclusion of up to 25% WCS in a complete ration for lactating cows actually improved digestibility of N, lipid and energy of the ration (Smith et al., 1981). There was a resultant increase in yields of milk fat and 4% fat-corrected milk. Increase in milk fat yield was apparently a result of direct transfer of dietary lipid to milk lipid. Fatty acid composition of the milk fat was more similar to the fatty acid composition of the WCS (predominantly C18) than normal milk fat of cows not fed WCS. Mammary synthesis of fatty acid also was reportedly reduced by 50% with 25% WCS in the diet compared with controls, as reflected in the reduced yields of C6 to C12 fatty acids. Their results agree with those reported in earlier studies of Astrup et al. (1976) and Smith et al. (1978).

Related studies involving feeding of whole sunflower seed (WSFS) have reported successful inclusion in rations of high producing dairy animals (McGuffey and Schingoethe, 1982; Rafalowski and Park, 1982). At low levels of inclusion in the diet (10 and 20% to 25% of the concentrate dry matter), WSFS did not cause any significant change in milk composition, nor was there any detectable difference in dry matter intake by animals. However, milk yields were higher for those receiving WSFS (Rafalowski and Park, 1982). In both studies, there was a greater proportion of C18 and lower proportions of C14 and C16 in the milk of cows fed WSFS compared with those not fed WSFS.

Higher proportions of long chain fatty acids, particularly stearate and oleate, have been found in milk of cows fed WCS (Smith and Collar, 1980). This may suggest some form of protection against ruminal microbial biohydrogenation of the polyunsaturated fats in WCS. It is unclear how polyunsaturated fatty acids in WCS escaped biohydrogenation in the rumen when WCS hardly could be found in contents of the reticulorumen or abomasum and in the feces (Anderson et al., 1982) of calves. Similarly, Coppock (1985) reported that less than 1% of dietary undelinted WCS appeared in the feces.

One might assume that the seed coat would be crushed during mastication. If such were the case, it would seem most unlikely that much fat would escape biohydrogenation by the ruminal microbial population. If, on the other hand, it is argued that the unsaturated fatty acids in WCS might actually have escaped ruminal biohydrogenation by virtue of the WCS's having passed through the forestomach unscathed, and getting digested in the abomasum and the intestines as suggested by Bath et al. (1980), it would seem that gossypol also would escape detoxification action of the rumen microbes. If this were true, it would seem possible that gossypol toxicity could be a problem to cows especially at high levels of intake of WCS. Smith (1983) reported that gossypol level in the blood of cows fed about 7.3 kg WCS/d was elevated to almost triple that of the control group, during the 15 wk of feeding WCS. None of the cows exhibited any sign of gossypol toxicity. Levels of up to 7.4 kg WCS/d (Smith, 1983) have been fed without any detrimental effects from gossypol. According to Lindsey et al. (1980), a limit gossypol tolerance could be expected when lactating cows are fed 10.2 kg CSM/d for an extended period which is equivalent to about 13.5 kg WCS/d.

Gossypol occurs freely in WCS constituting between .4 to 1.4% of the weight of the kernel (Gohl, 1981). It is toxic to animals if fed in large quantities. Processing of seeds with a screw press into meals, however, causes much of the compound to be converted into bound forms thus lowering its biological activity (Jones, 1981). Solvent processes do not have similar effects.

Deleterious effect of gossypol is severe especially in the nonruminants. Ruminant animals are protected apparently from the adverse effects of gossypol by the fermentative activity of the ruminal microbes (Reiser and Fu, 1962). Evidence in support of this is grossly lacking. On the contrary, a recent study by Lindsey et al. (1980) indicated that there is a limit to the capacity of the rumen to detoxify gossypol in the feed. Further, it was observed that consumption of excessive amounts of free gossypol could have a detrimental effect on the performance of a cow, particularly if she is under high milk production and other forms of stress. One cow on a solvent-extracted CSM ration for 81 d died. Her estimated daily consumption of gossypol was 8 mg free and 222 mg total gossypol/kg body weight. Smith (1983) detected elevated blood gossypol with WCS feeding at 7.3 kg daily and cautioned that, if sustained for an extended period, it could lead to gossypol toxicity. Susceptibility of ruminants to high gossypol in WCS diet cannot be completely dismissed as yet.

A more recent study on possible gossypol toxicity in cattle was by Coppock et al. (1985b) in which 47 cows (20 lactating and 27 pregnant nonlactating) in two experiments were fed high levels of WCS. Responses monitored were intake, rate of respiration and concentration of blood metabolites. The first experiment involved 20 lactating cows given diets containing WCS at 0, 15 or 30% of diet dry matter in a 50:50 corn silage:concentrate diet. The second involved 27 nonpregnant dry cows fed diet of 65:35 corn silage:concentrate ratio. Three levels of WCS at 15, 35 and 55% of diet dry matter were included by substituting for equal portions of the concentrate. Although dry matter intake declined with 30% WCS diet in experiment 1 and 35 and 55% WCS in experiment 2, other measured responses were not indicative of possible gossypol toxicity. Intakes of WCS dry matter were 5.17 and 5.53 kg/d for the 35 and 55% WCS diets, respectively. Authors suggested that gossypol toxicity was unlikely because cows could self-limit intake of high WCS diets. Moreover, high dietary Ca and Mg could have caused binding of gossypol (Coppock et al., 1985b).

Supplemental Proteins for Ruminants

Protein in the diet is important as a source of amino acids which are necessary for various essential synthetic processes in the body. In ruminants, the amino acids required may be supplied directly in the diet, in the rumen microbes or in the endogenous secretions from normal turnover of body tissues (Chalupa, 1975; Satter and Roffler, 1975). There is limited storage of proteins in tissues (Platt et al., 1964) confined specifically in the blood, liver and muscle. These do not form adequate reserves to overcome deficiency periods during pregnancy and lactation.

Generally for ruminants, requirements for amino acids are met from ruminal microbial supply and from dietary proteins that escape ruminal degradation and get digested and absorbed in the intestine. Ruminal microbial protein alone in most cases cannot provide enough protein to meet requirements for maximum production (NRC, 1985). Without a supply of ruminal undegraded protein in the diet, growth and/or production may be limited.

An excess of protein in the diet is wasteful and costly, particularly if the protein supplied is of the kind that is rapidly degraded in the rumen. Feed protein as well as nonprotein nitrogen (NPN) may be degraded rapidly in the rumen with release of ammonia. About 60% of feed protein entering the rumen is degraded by ruminal bacteria and only 40% goes to the abomasum unchanged (Van Horn and Swanson, 1978). If ammonia production exceeds the capacity of the bacterial population to use ammonia, some of the excess may be lost (NRC, 1985), and at very high concentrations it can cause toxicity. Ammonia and other NPN fractions not incorporated into bacterial protein are of little nutritional value to the animal. For extensive reviews of ruminant nitrogen metabolism, readers are referred to Huber and Kung (1981) and Leng and Nolan (1982).

Sources of Supplemental Proteins

Soybean meal and cottonseed meal are two traditional supplements that have been used widely in livestock diets. Van Horn (1982) presented a treatise of newer sources of proteins for ruminants which include whole oilseeds and meals derived from sunflower, peanut, cottonseed and soybean industries, and by-products from the brewery and distillery such as brewer's grains and distiller's grains. These are excellent sources of proteins for the ruminants both from the standpoint of their amino acid profile and their resistance to ruminal microbial degradation. Soybean meal is widely used because of its availability.

Bypass Characteristics of Natural Proteins

Feedstuffs that are selected more commonly in the United States for their characteristics of escaping degradation in the rumen include brewer's dried grains, distiller's dried grains, corn gluten feed and meal, and dehydrated alfalfa meal (Macleod and Grieve, 1983). Estimated rumen degradability values of 40% were reported for this group of feedstuffs. The NRC (1985) estimated 47, 45 and 38% ruminal protein degradability of brewer's dried grains, corn gluten meal and distiller's dried grains, respectively. Ruminal degradability values could vary with level of intake, feed processing and other factors (Zinn et al., 1981; Satter and Stehr, 1984). The more conventional protein supplements, soybean meal, peanut meal and cottonseed meal, have 60% or higher degradability values.

With the exception of dehydrated alfalfa meal, feedstuffs with lower degradability values are all of grain origin. Proteins in grains are less readily degraded in the rumen (Klopfenstein et al., 1978, 1980). In addition, heating, that is applied to grains during the drying process, most likely has contributed to the amount of ruminally undegradable protein in these by-product feedstuffs (Klopfenstein et al., 1976).

Klopfenstein et al. (1978) presented results from a series of experiments evaluating distiller's grain products as a source of naturally-protected protein supplements for ruminant diets based on 60% corn cobs and 10% molasses. These diets were compared with urea- and soybean meal-supplemented diets by measuring liveweight gains of calves and by digestion and metabolism trials with lambs. Average daily gain on milo DDG plus urea was nearly identical to soybean meal and was superior to both milo DDG plus solubles plus urea and diets supplemented only with urea. A different experiment compared corn DDG with corn DDG plus solubles and with soybean meal, each supplemented with urea to provide 60% of dietary crude protein. Superior average daily gain and efficiency of feed utilization were noted with DDG diets. Also, all diets resulted in similar dry matter and nitrogen digestibilities.

Satter and Stehr (1984) summarized results of studies comparing effects of DDG as a low rumen-degradable protein supplement versus SBM on milk yield and composition. They reported a 2.7% improvement in milk yield to DDG- over SBM-supplemented diets. However, the trend was not evident in another experiment in which they used either extruded mixture of soybeans plus SBM, or DDG plus solubles plus CGM, or a 50:50 combination of these as low rumen-degradable protein supplements as compared with conventional SBM-supplemented diets. Milk yields from both diets that contained DDG were lower than the SBM control. Idaho workers (Roffler et al., 1984) reported no difference in milk yield from diets supplemented with CGM or SBM.

Supplementation of diets with low rumen degradable proteins affect milk composition in many of the studies reviewed. Data of Palmquist and Conrad (1982) showed indication of depression of milk protein but elevation of milk fat in both Jersey and Holstein cows receiving DDG plus solubles as supplemental protein versus SBM. Similar trends were evident from data of Roffler et al. (1984), which compared CGM to SBM, and of Satter and Stehr (1984), which showed a reduction in milk protein over SBM control when supplemental protein was from either extruded mixture of soybean plus SBM or a mixture of DDG plus solubles plus CGM.

Current emphasis of nutritionists is on minimizing losses of dietary protein accruing from microbial degradation in the rumen. This can be achieved through proper selection and balance of the feed ingredients in diet formulations or use of protected proteins. The former approach takes advantage of the fact that feedstuffs vary widely in degradability. Inclusion in the diet of proteins that escape ruminal degradation and some highly soluble proteins or NPN which supply ammonia for optimum microbial growth is being given close attention (Van Horn, 1982).

Estimation of Digestibility of Feeds

In the context of animal nutrition, digestibility refers to the percent of the feed or of a single nutrient or component in the feed that is dissolved, acted upon and degraded in the digestive tract so that it can be absorbed and beneficially used by the organism (Schneider and Flatt, 1975). Conventionally, digestibility of a feed is estimated in a digestion trial by live animal experimentation involving total collection of feces. A detailed procedure for a digestion experiment is as described by Schneider and Flatt (1975).

Basically, a digestion trial involves measuring the amount of feed consumed and the amount excreted in the feces during a definite time period. From analysis of the nutrients in the feed and feces, the digestibility of a nutrient can be calculated by difference, which represents the fraction of the nutrient in the feed that cannot be accounted for between the point of intake and the point it is voided in the feces. The value obtained is more appropriately termed the apparent digestion coefficient because the feces also contain metabolic products such as microbial cells and endogenous materials in addition to the undigested intake materials (Van Soest, 1983).

Estimation of digestibility by total collection of feces is laborious, costly and a time-consuming procedure. For this reason, various methods have been proposed and tested as an alternative method of assessing digestibility. While this eliminates the necessity for total fecal collection, no one method gives a better estimate of digestibility than the total collection method. The more common of these are the in vitro technique, the digestive marker ratio technique and the in situ technique.

In Vitro Technique

The in vitro technique, in essence, is an artificial rumen system outside the animal. Basically, it is a vessel inside which the rumen environment is simulated. Innoculum supplying the microbial population normally is supplied in the form of strained rumen fluid. The medium is usually a buffer solution simulating saliva of the animal. A small amount of the diet or feedstuff for which digestibility is to be estimated is added to the system in an anaerobic condition at ruminal temperature (39 C). While the normal practice is to allow an incubation time of 48 h for estimation of extent of digestibility, this can be varied according to need and purpose.

One of the most successful and widely used in vitro systems is that which is based on the procedure developed by Tilley and Terry (1963). Oh et al. (1966) concluded that, for all types of forages, dry matter digestibility is best predicted by the Tilley and Terry (1963) procedure. Work of Johnson (1969) comparing 77 forages subjected to several in vitro analyses provides support for the conclusion of Oh et al. (1966).

Definite advantages exist for in vitro systems over other systems. Firstly, unlike total collection, in vitro system requires small samples and is easily adaptable to handle a large number of feeds for screening purposes (Johnson, 1969; McCleod and Minson, 1969). It has the potential of being more accurate than chemical systems because of presence of microorganisms which are sensitive to undetermined factors that influence rate and extent of digestion (Van Soest, 1983). It provides a relatively good prediction of feed quality, whereas to obtain a reasonably accurate estimate by a chemical method would require more than one analysis (Van Soest, 1983). Modifications of the Tilley and Terry procedure (1963) have been proposed and tested (Moore and Mott, 1974), and this has produced results that are correlated highly with in vivo data.

Digestibility Marker Ratio Technique

This method, also often referred to as indicator method, involves use of a substance meeting certain criteria. Faichney (1975) described the ideal marker as one that is nonabsorbable and does not affect nor get affected by the digestive process. In addition, it must be similar physically to or be associated with the material it is to mark and it must have a specific and sensitive method of estimation in the digesta samples.

Markers for digestion studies may be classified into two general groups: internal and external. The former refers to those that are natural constituents of the feeds. Among those that have been investigated are silica, plant chromogens, lignin, acid insoluble nitrogen and acid insoluble ash (Thonney, 1981; Thonney et al., 1985). Acid detergent fiber frequently has been used to estimate relative digestibility (Marten, 1981). External markers, on the other hand, are extraneous substances that are added to the diet during the period of the experiment. These include powdered cork, sulfides of heavy metals, ferric oxide and chromic oxide (Thonney, 1981). In a review on the use of markers (internal and external) in digestibility studies as compared to total fecal collection, Kotb and Luckey (1972) concluded that use of marker methods offer the advantages of cheapness and convenience. However, they cautioned against use without precautions.

Internal markers have an advantage in that they require no mixing into the diet as do external markers. Acid insoluble ash and lignin are the two more popular internal markers in digestion trials with ruminants (Van Keulen and Young, 1977; Muntifering, 1982; Fahey and Jung, 1983; Thonney et al., 1985). Acid detergent fiber and lignin assays have been used in estimating relative digestibilities (Marten, 1981; Hunt et al., 1984). Dry matter digestion coefficients were similar to total collection (Hunt et al., 1984).

Acid insoluble ash is of particular interest as an internal marker because of relatively simple determination procedure. Van Keulen and Young (1977) evaluated several procedures for determination of acid insoluble ash differing in ashing sequence, ashing temperature and acid strength. They found that the procedure using 2N HCl was the most convenient and most rapid as well as the safest compared to concentrated or 4N HCl. Thonney et al. (1985) improved on the procedure of Van Keulen and Young (1977) by reducing sample ashing to once and eliminating the need to transfer the ash. Accuracy of digestion coefficient estimations by the acid insoluble ash method is reportedly good. Shrivastava and Talapatra (1962) used it as a marker in sheep experiment and reported average residue recovery of 99.8%. Estimated digestibility coefficients were not different from values obtained by total collection method. Van Keulen and Young (1977) similarly reported marker recovery not significantly different by any of the acid strengths used. Estimates of dry matter digestibility were satisfactory. Results of Thonney et al. (1979) further validated the above findings. High marker recoveries ranging from 90 to 106% were reported even for a 80% concentrate ration. Dry matter digestibility was similar to that estimated by total collection.

Even with such high precision compared with total collection, use of acid insoluble ash as a digestibility marker has to be considered with care. Most important is getting a representative sample of the material consumed and of the feces (Van Keulen and Young, 1977). There is indication that acid insoluble ash serves best as marker where diets are high in the constituent. Thonney et al. (1985) suggested only when diets contained 0.75% or more acid insoluble ash in the total dry matter could it be used with confidence as a digestibility marker. Its use may be limited where possibility of ingesta contamination with sand or soil is high (Van Keulen and Young, 1977) which possibly explains the poor agreement of digestibility estimates determined by total collection or acid insoluble ash by Romero (1985).

Use of lignin as a marker in digestion experiments has been extensive. Its reliability as a marker has been subject of much controversy because of the question of whether or not it is digested and absorbed in the digestive tract. A recent review by Fahey and Jung (1983) presented much of the findings that have accumulated. Digestibility of lignin varies between -5.1 and 23.7% (Fahey and Jung, 1983). Permanganate lignin had apparent digestibilities ranging from 27.9 to 53.3% (Galyean et al., 1979). The high apparent digestibility of lignin caused a 23.9% underestimation of digestibility values by the permanganate lignin ratio technique compared with total collection (Thonney et al., 1979). According to Van Soest (1983), poor fecal lignin recovery and consequent poor estimates of digestibility coefficients most likely are attributed to lack of accurate method of its identification. Fixed time oxidation (90 min) as set out in the procedure for permanganate lignin may be inadequate for samples high in lignin such as fecal samples.

Claims of partial digestion of lignin along the gastro-intestinal tract have appeared in the literature. Reports of possible true digestion were by Allinson and Osbourn (1970) and Porter and Singleton (1971) and of apparent digestion (Fahey et al., 1979) through formation of soluble lignin-carbohydrate complexes that pass out of the rumen, and presumably also the abomasum, as polymers not recovered in the fecal fiber fraction. While there is report of aerobic degradation of both simple phenolic monomers and lignin by soil fungi and bacteria (Crawford and Crawford, 1980), true digestion of lignin has not been demonstrated in the ruminant. For this reason, lignin is considered indigestible (Bellamy, 1974; Van Soest, 1983) by anaerobic microbes or associated digestive enzymes in the ruminant.

Akin (1980) succeeded in isolating from rumen fluid a filamentous, facultative anaerobic bacteria capable of degrading lignified tissues. It is doubtful that these organisms contribute significantly to lignin digestion in the rumen because of their relatively small numbers and slow growth rate. Nevertheless, these organisms may have potential contribution in degrading lignin in in vitro and in vivo systems.

Muntifering (1982) evaluated various lignin assays (acid detergent lignin, permanganate lignin and acetyl bromide lignin) as markers for ruminal digestion estimates in lambs fed diets containing tall fescue, corn cobs or CSH. He reported that estimates of ruminal organic matter digestibility by the acid detergent and permanganate lignin methods were comparable to that estimated using chromic oxide marker. Acetyl bromide soluble lignin tended to overestimate ruminal digestibility. Mean fecal recoveries of the markers were 97.5, 78.0, 78.4 and 65.0% for chromic oxide, acid detergent lignin, permanganate lignin and acetyl bromide soluble lignin, respectively. Van Soest

(1983) suggested that use of lignin as an indigestible marker should be limited to diets that contain over 7% lignin in the dry matter.

Among external markers for estimating digestion, chromic oxide is the one used most commonly (Van Soest, 1983). It is a fine, heavy, insoluble powder, green in color. According to Kotb and Luckey (1972), chromic oxide powders pass more rapidly from the rumen than coarse fibers of feeds and tend to move with the liquid fraction. Like all external markers, it is essential that chromic oxide be mixed with the ingesta or feed to attain an even distribution of the powder which should contribute to its even passage down the digestive tract. External markers, in general, may lack the capacity to stay with the feed along the digestive tract and often are reported to exhibit a diurnal pattern of excretion thus contributing to the problem of incomplete recovery in the feces (Prigge et al., 1981; Thonney et al., 1985).

Twice-daily dosing has reduced diurnal variation (Prigge et al., 1981) considerably. The practice of fecal sampling by taking random grab samples from the rectum likewise has kept such variation lower and thus precludes the need for total collection of feces in digestibility experiments (Prigge et al., 1981).

In Situ Technique

In situ means in place or original position. Earliest reported study using the principle of the in situ technique was by Raeumur in 1752, cited by Wilson and Strachan (1981), in which digestibility of leaves was studied by placing them in a perforated brass capsule administered to sheep. In 1938, Quin et al. improvised on the technique by incubating feedstuffs in small cylinderical silk bags in the rumen of fistulated sheep (Orskov, 1982). Many later in situ experiments used artificial fibers in substitution for silk as material for the bags. Preference for the former lies in the fact that they are resistant totally to ruminal degradation by the microbes (Figroid et al., 1972). Dacron polyester bags offer yet another advantage having lower nitrogen content (Crawford et al., 1978) compared to nylon (0.59 versus 12.4% N), particularly for studies of nitrogen degradability in the rumen.

Basically, the in situ technique involves placing a known quantity of feedstuff, usually ground to 2 mm in size, into small bags and the entire bags are incubated inside the rumen by passage via a fistula. Bags are withdrawn after different incubation intervals, washed and dried in the oven. The difference in weight of the feed pre- and postincubation constitutes the fraction of the feed or nutrient that has been degraded and presumed digested in the rumen.

The attractiveness of in situ method for estimating digestion lies in the ability to follow sequentially the rate of degradation (Wilson and Strachan, 1981). Also, larger numbers of different raw materials or feedstuffs can be processed simultaneously than with the traditional in vivo method, requiring only a simple rumen cannulation. Therefore, the in situ technique appears to be an appropriate tool for use in the less developed countries in feed evaluation programs provided that attention is given to the factors causing variation in results as discussed by Nocek (1985). These include order of bag introduction, animals used, incubation intervals, contamination from microbial N, feed particle size, ratio of sample weight to bag surface area and pore size of bag.

Generally, there has been good agreement between data for feed protein degradability obtained by in vivo and in situ methods. Evidence is from data of Mathers and Miller (1981) who compared degradability of protein from lucerne and rolled barley using sulfur as microbial marker in in vivo and in situ degradation. Similarly, Zinn et al. (1981) reported close agreement between degradability values in situ and in vivo using nucleic acid as the microbial marker.

CHAPTER III
EFFECTS OF DIETARY WHOLE COTTONSEED, ROUGHAGE SOURCE AND ADDED LIMESTONE ON LACTATIONAL PERFORMANCE AND DIETARY FIBER DIGESTIBILITY

Introduction

Submaximal intake is a major factor limiting maximum milk yield in lactating dairy cows. This holds true particularly when feeds that are relatively low in nutritive value constitute a major portion of the cow's diet. Intake of cows consuming such diets most likely will be limited by physical fill or ruminal distension (Montgomery and Baumgardt, 1965). Limited in their ability to match their higher nutrient demand of early lactation with increased feed intake, cows mobilize energy reserves from body fat depots and invariably go into negative energy balance (Coppock, 1985).

High producing dairy cows usually are fed liberal amounts of concentrates to increase the energy content of the diet (Elliot and Bauman, 1980). Supplementation with fats or oils was suggested as an alternative method of increasing energy density (Palmquist and Jenkins, 1980), thereby increasing energy intake. One of the most convenient ways of adding fats to the diet is through addition of whole oil seeds because no additional processing is required. There likely is less interference with fiber digestion by the fat added in this way (Van Horn, 1985).

Use of whole cottonseed (WCS) for feeding higher producing cows is a common practice and is becoming increasingly popular among dairy farmers in the United States (Smith et al., 1981). Value of WCS is associated with a number of its nutritive and physical attributes including a high concentration of energy in the oil fraction, a relatively good quality and high quantity of protein and a highly digestible fiber in the lints which constitute about 10% of the weight of linted WCS (Coppock et al., 1985a).

Increased milk yield (Anderson et al., 1979; Van Horn et al., 1984) but decreased milk protein percent (Anderson et al., 1979; Smith and Collar, 1980) have been reported with WCS feeding (up to 30% of diet dry matter). However, the change in milk fat percent has not been consistent always. Some dairymen in Florida have experienced no increase in milk fat test from WCS inclusion in diets based on either corn silage (CS) or cottonseed hulls (CSH) (Beede, personal communication). Furthermore, Van Horn et al. (1984) reported reduced milk fat percent when WCS was fed in diets equal in crude fiber content to control diets containing no WCS. Sources of roughage were cottonseed hulls, steam pressure-treated bagasse pellets and CS.

It was possible that the variability in responses was related to the high oil content of WCS. High fat-supplemented diets depressed fiber digestion (Johnson and McClure, 1973; Jenkins and Palmquist, 1982) and, as a consequence, milk fat content could be reduced. With high dietary calcium, however, it may be possible to alleviate partially the negative inhibitory effects of fat on fiber digestion

(Jenkins and Palmquist, 1982). Calcium can form insoluble soaps with fatty acids intraruminally, reducing potential inhibitory effects of free fatty acids on cellulolytic bacteria in the rumen. Improvements in fiber digestibility have been associated with calcium additions from various sources. Brooks et al. (1954) reported effectiveness of alfalfa ash in alleviating depressed fiber digestion due to corn oil. The ash was quite high in calcium. In a California study, WCS addition to a diet with 48% alfalfa hay showed no evidence of milk fat depression (Smith et al., 1980). The hay contributed about 95% of the total dietary calcium and the total diet contained .68% calcium, dry basis.

Cottonseed hulls (CSH) as a roughage source in lactation diets was studied (Vernlund et al., 1980; Harris et al., 1983; Van Horn et al., 1984). Dry matter digestibility of diets based on 30% CSH was low (47%) (Vernlund, 1979). However, milk yield responses to CSH-based diet were favorable compared with the more conventional roughage source, CS (Harris et al., 1983); cows produced 26.4 and 24.4 kg/d with CSH- and CS-based diets, respectively. Milk fat percent also was higher for CSH-based diets than for CS-based diets (3.44 vs. 3.25%). These differences were attributed to the greater feed intake of CSH-based diets (Harris et al., 1983).

Van Horn et al. (1984) compared CSH with other unconventional sources of roughage, pelleted steam pressure-treated bagasse and ground corrugated boxes, in diets also containing WCS. Dry matter intake was higher consistently for CSH-based diets than for diets based on the other roughages. They suggested possible interactive effects of WCS and roughage sources on dry matter intake and milk yield.

The primary objective of the present experiment was to evaluate effects of three dietary levels of undelinted WCS and added limestone in diets based on CS, CSH or AH on feed intake, milk yield, milk composition and fiber digestibility. Of particular interest were potential interactions of WCS with roughage sources. A secondary objective was to assess potential relative differences in dry matter intake and milk yield due to roughage source when equal amounts of the total diet dry matter were contributed by CSH, CS or AH.

Materials and Methods

Dietary Treatments

Dietary treatments were arranged in a 3x3x2 factorial of WCS (0, 15 and 30% of diet dry matter), roughage sources (CS, AH or CSH) and added limestone to give .69 or 1.29% total dietary calcium, dry basis (table 1). Roughage content of each diet was fixed at 40% of total diet dry matter. Proportions of other dietary ingredients used are in table 2.

Experimental diets (table 2) were formulated using typical feedstuff compositions (NRC, 1978). Individual concentrate mixes were prepared in 909 kg batches and kept in separate storage bins for daily feeding. Total mixed rations were prepared daily. Appropriate portions of roughage, WCS and respective concentrate mix were weighed into a modified cement mixer for mixing and then fed as a complete mixed ration to each cow.

Table 1. Dietary Treatment Arrangement

Roughage[b] source	Levels of whole cottonseed[a]					
	0		15		30	
	Total dietary calcium,%[c]		Total dietary calcium,%		Total dietary calcium,%	
	.69	1.29	.69	1.29	.69	1.29
Corn silage	1[d]	10	4	13	7	16
Alfalfa haylage	2	11	5	14	8	17
Cottonseed hulls	3	12	6	15	9	18

[a] Levels are percent of total diet dry matter.

[b] Roughage sources constituted 40% of total diet dry matter.

[c] Additional calcium provided from feed grade limestone.

[d] One through 18 are dietary treatment numbers.

Table 2. Ingredient Composition of Diets

Ingredients	Diet number																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Corn silage	40.0[a]	-	-	40.0	-	-	40.0	-	-	40.0	-	-	40.0	-	-	40.0	-	-
Alfalfa haylage	-	40.0	-	-	40.0	-	-	40.0	-	-	40.0	-	-	40.0	-	-	40.0	-
Cottonseed hulls	-	-	40.0	-	-	40.0	-	-	40.0	-	-	40.0	-	-	40.0	-	-	40.0
Whole cottonseed	-	-	-	15.0	15.0	15.0	30.0	30.0	30.0	-	-	-	15.0	15.0	15.0	30.0	30.0	30.0
Crimped corn	35.5	46.3	29.2	26.1	39.1	20.2	17.6	28.5	11.3	33.2	44.7	28.5	24.4	36.0	18.6	15.9	26.8	9.6
Soybean meal (49% CP[b])	21.0	12.0	27.8	15.5	5.3	21.9	9.1	-	16.0	21.6	11.9	27.8	15.5	5.7	21.9	9.1	-	16.0
Trace mineralized salt[c]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	.3	.4	.4	.2	.2	.2	-	-	-	.3	.4	.4	.2	.2	.2	-	-	-
Potassium chloride	.4	-	.6	.3	-	.5	.3	-	.5	.4	-	.6	.3	-	.5	.3	-	.5
Magnesium oxide	.3	.2	.1	.3	.1	.1	.2	.1	-	.3	.2	.1	.3	.2	.1	.2	.1	-
Limestone	1.0	1.0	.9	1.1	.3	1.1	1.3	.4	1.2	2.7	1.8	2.6	2.8	1.9	2.7	3.0	2.1	2.9
Potassium sulfate	.5	-	-	.5	-	-	.5	-	-	.5	-	-	.5	-	-	.5	-	-

[a] Values calculated on percent, dry matter basis.

[b] CP = Crude protein.

[c] Trace mineralized salt contains 95-99% NaCl, .35% Zn, .34% Fe, .20% Mn, .033% Cu, .007% I and .005% Co.

Animals and Management

Thirty-six mid-lactation Holstein cows were used. Cows were housed in a free-stall barn. Each pen accommodating 12 cows was equipped with four shared automatic waterers and feed bunks for individual cows had controlled access by electronic gate devices (American Calan, Inc., Northwood, NH). Individual cow feed intake was measured daily. Cows were kept in their respective pens throughout the experiment except at milking and were allowed access to dirt lots adjoining the pens for about 2 h daily prior to evening milking. Milking was twice daily at 0630 to 0730 h (morning milking) and at 1900 to 2000 h (evening milking).

Cows were fed a standardization diet (diet 1) for 2 wk before being offered their respective treatment diets at the beginning of period 1. Fresh feed was offered to each cow once daily and adjustments in amount of feed offered were made each day to allow for about 10 to 15% refusals (as fed). Feed refusals from each cow were collected each morning and weighed. Amount consumed daily was calculated by difference. Dietary treatments were switched abruptly one day to the next, from one period to the next. Animals were weighed once after the morning milking at the beginning of the experiment and at end of each period.

Design and Statistical Analysis

Dietary treatment sequences were assigned randomly to cows (table 3) in a partially balanced incomplete block design. Assignment of treatments were such that each cow received a different dietary treatment in each period, and no treatment followed another treatment

Table 3. Dietary Treatment Sequence Assignment

	Period		
Cow	1	2	3
637	1[a]	14	9
655	7	15	2
657	3	13	8
713	5	18	1
744	9	14	1
764	7	11	6
766	11	9	13
781	9	10	5
791	13	2	18
793	15	1	17
794	17	6	10
796	18	4	11
840	11	4	18
848	15	8	10
853	12	5	16
864	10	6	17
888	16	3	14
910	8	12	4
935	14	3	16
939	8	13	3
945	6	16	2
956	4	17	3
959	16	5	12
961	2	15	7
965	14	7	12
966	1	18	5
967	3	17	4
968	12	7	14
978	10	8	15
987	5	10	9
1004	13	9	11
1018	17	1	15
1024	6	11	7
1035	4	12	8
1037	2	16	6
1056	18	2	13

[a] Diet numbers.

more than once during the experiment. In all, 108 cow-period observations were possible.

Milk production and feed intake data from the last 14 d of each of the three 35-d periods were analyzed by method of least squares analysis of variance using general linear model procedures of the Statistical Analysis System (SAS, 1982). The mathematical model was cow, period, treatment (table 6).

Sampling and Chemical Analyses

Milk samples were collected from the last six milkings of each period. About 50 ml samples were taken at each milking in plastic bags containing potassium dichromate tablets as a preservative. Each day's samples (morning and evening milkings) were pooled and sent to the North Carolina Dairy Herd Improvement Laboratory, Raleigh, NC, for analyses of milk protein, milk fat and somatic cell count.

Samples of AH and CS were collected every third day at feeding and analyzed for dry matter to make necessary adjustments in the roughage to concentrate proportions (dry basis) for daily rations. Random samples of concentrate mixes and CSH were taken when each new batch of concentrates was prepared. Dry matter was determined by drying in a forced-draft oven at 60 C for 48 h. Samples were then stored refrigerated (4 C) for future analyses. At the end of each period, samples were pooled within roughage source or individual concentrate mix. The roughages were ground in a Wiley mill (2 mm screen). A representative sample of each was taken for chemical analyses. Feed samples of each of the concentrate mix, roughage sources and WCS were sent to New York Dairy Herd Improvement Forage Testing Laboratory, Ithaca, NY, for chemical analyses.

Digestibility Estimation

Estimates of digestibilities of dry matter (DM), acid detergent fiber (ADF) and neutral detergent fiber (NDF) for each dietary treatment were determined using the internal marker permanganate lignin. These estimates were to provide relative rankings of the effects of dietary treatments in digestion. Fecal grab samples were collected twice daily following milking on the final 3 d of each period. Samples were kept frozen (-15 C). At the end of each period, samples were thawed and composited by cow-period. Fecal dry matter was determined by drying in a forced-draft oven at 60 C for 48 h. After allowing samples to equilibrate at room atmospheric conditions for 2 d, samples were ground in a Wiley mill (2 mm screen). Ground samples were kept in plastic bags for subsequent chemical analyses.

Neutral detergent fibers, ADF and permanganate lignin contents of the feed ingredients and fecal samples were determined using procedures of Goering and Van Soest (1970) with some modification. In the NDF determination, gooch crucibles lined with glass wool were used (as per J. E. Moore, personal communication). Starting with the same original sample, ADF and permanganate lignin determinations were carried out in scintered glass crucibles. A known quantity of solka floc with a known disappearance in the procedure was added to each of the ADF samples prior to digestion to serve as a filtering aid in the ADF and permanganate lignin determinations. Cellulose was estimated by difference in weight between residues after permanganate oxidation and after ashing. Each analysis was in duplicate and an experimental error of less than 5% was accepted before re-analysis was considered necessary.

Results and Discussion

Chemical Composition of Dietary Treatments

Partial chemical compositions of the total mixed diets are in table 4. Values were obtained by calculations using actual chemical analyses of WCS, the respective roughage sources and concentrate mixes according to the proportions described. Average chemical analysis of WCS and of each roughage source are in table 5. Whole cottonseed contained 23.7% crude protein (CP) and 35.1% ADF which were in close agreement with values of Bernard and Amos (1985) who reported 23.2 and 37.7%, CP and ADF, respectively. Overall, analyses of the roughage sources and WCS (table 5) were comparable with those of the NRC (1982). Overall analyses of each dietary treatment are listed in appendix table A-1.

One of the objectives of this experiment was to evaluate the effects of the roughage sources on total mixed rations consumption; therefore, their levels in the dietes were equalized (40% of the diet dry matter). Consequently, energy content of treatment diets varied. Corn silage diets were highest in net energy for lactation (NE_l) (1.7 to 1.8 Mcal/kg) compared with AH- and CSH-based diets which ranged from 1.5 to 1.7 Mcal/kg and 1.4 to 1.6 Mcal/kg, respectively (table 4). Variations in NE_l contents of dietary

Table 4. Chemical Composition of Diets

Diet	Roughage source	NE_1[a,b]	CP[a]	ADF[a]	Ca[a]	P[a]
		(Mcal/kg)	-------------	-------------	(%)	-------------
1	Corn silage	1.7[c]	18.7	14.7	.80	.41
10	"	1.7	18.3	14.4	1.49	.38
4	"	1.8	17.8	19.0	.93	.41
13	"	1.8	17.7	19.7	1.34	.37
7	"	1.8	17.1	23.3	.83	.40
16	"	1.8	17.0	23.1	1.21	.38
2	Alfalfa haylage	1.5	18.0	20.8	.57	.47
11	"	1.5	17.2	20.8	1.31	.45
5	"	1.6	16.7	25.3	.64	.47
14	"	1.6	16.3	25.5	1.29	.44
8	"	1.7	16.3	29.7	.63	.46
17	"	1.7	16.1	29.8	1.50	.44
3	Cottonseed hulls	1.4	20.2	30.9	.60	.37
12	"	1.4	19.7	30.9	1.11	.34
6	"	1.5	19.2	35.0	.67	.35
15	"	1.5	19.0	36.7	1.13	.33
9	"	1.6	18.9	39.5	.62	.35
18	"	1.6	18.1	39.4	1.23	.32

[a] NE_1 = Net energy (lactation); CP = Crude protein; ADF = Acid detergent fiber; Ca = Calcium; P = Phosphorus.

[b] Calculated based on chemical analyses.

[c] Based on calculations from actual analyses. Values on dry matter basis.

Table 5. Average Analyses of Roughages and Whole Cottonseed

Item	Dry matter (%)	NE_1[a] (Mcal/kg)	Percent of dry matter							
			CP[a]	TDN[a]	ADF[a]	Ca[a]	P[a]	Mg[a]	K[a]	Na[a]
Corn silage	29.1	1.56	8.3	69.0	28.4	.59	.26	.18	1.38	.02
Alfalfa haylage	32.0	1.06	16.3	55.0	45.9	1.00	.44	.45	2.12	.04
Cottonseed hulls	94.7	.98[b]	4.2	45.0[b]	67.6	.12	.05	.12	1.00	.01
Whole cottonseed	95.5	2.23[b]	23.7	96.0[b]	35.1	.13	.62	.35	1.12	.01

Note: Composite of samples taken throughout the experiment.

[a] NE_1 = Net energy lactation; CP = Crude protein; TDN = Total digestible nutrient; ADF = Acid detergent fiber; Ca = Calcium; P = Phosphorus; Mg = Magnesium; K = Potassium; Na = Sodium.

[b] From feed composition tables (NRC, 1982).

treatments containing the same roughage source were due to varying levels of inclusion of WCS. The variation in ADF content among diets was largely a reflection of differences in the ADF content of the constituent roughage sources (table 5). For CS-based diets, average ADF was 19.0% compared with 25.3 and 35.4% for AH- and CSH-containing diets, respectively. Within dietary treatments composed of the same roughage source, the difference in ADF content of the total mixed diets was due largely to varying WCS addition.

Effects of Dietary Treatment on Production Variables

Except for two cows which had trouble adjusting to the abrupt switch in dietary treatment from one period to another, there was no appreciable problem with feed intake. Even for those two cows, it only took between 7 to 10 d before they were consuming normal amounts of the new dietary treatment. One cow required treatment for milk fever, presumably as a result of abrupt switch-over from a high calcium (1.29%) to a lower calcium diet (.69%) from period 2 to 3.

Least-squares analyses of variance and least-squares means of dietary treatment effects on lactational performances are in tables 6, 7 and 8. Least-squares means for individual dietary treatments for all dependent variables are in appendix table A-2. For most of the dependent variables studied, cow and period effects were significant. There were dietary treatment effects on all production variables studied. Overall, feed dry matter intakes were similar in all three periods (22.5, 23.2 and 22.0kg/d for periods 1, 2 and 3).

Effects of dietary whole cottonseed level. Level of dietary WCS addition affected daily dry matter intake (tables 6 and 8).

Table 6. Least-Squares Analysis of Variance of Dry Matter Intake, Milk Yield, Milk Fat Percent and Milk Fat Yield

Source	df^a	Dry matter intake (kg/d)		Milk yield (kg/d)		Milk fat (%)		Milk fat yield (kg/d)	
		MS^a	P>F^a	MS	P>F	MS	P>F	MS	P>F
Cow	35	26.111	.0001	48.527	.0001	.503	.0001	.032	.0001
Period	2	11.999	.1195	40.323	.0001	.372	.0023	.019	.0102
Whole cottonseed (W)	2	118.344	.0001	15.831	.0005	.613	.0001	.082	.0001
Linear (W_L)	1	229.346	.0001	12.295	.0108	1.170	.0001	.125	.0001
Quadratic (W_Q)	1	8.718	.2102	19.861	.0015	.063	.2844	.042	.0014
Calcium (Ca)	1	2.867	.4700	.172	.7553	.015	.5993	.001	.9148
Roughage (R)	2	77.390	.0001	5.131	.0629	.171	.0505	.001	.8473
$C_1{}^b$	1	140.963	.0001	.563	.5737	.001	.9936	.001	.9835
$C_2{}^b$	1	13.585	.1194	9.706	.0226	.343	.0150	.001	.5670
$C_3{}^b$	1	71.085	.0007	9.436	.0245	.258	.0336	.001	.6272
$C_4{}^b$	1	83.547	.0003	.831	.4944	.085	.2152	.001	.7603
W x Ca	2	25.653	.0130	2.051	.3189	.042	.4671	.001	.9263
W_L x Ca	1	4.961	.3429	.130	.7867	.015	.6059	.001	.7015
W_Q x Ca	1	45.894	.0053	3.993	.1376	.068	.2672	.001	.9495
W x R	4	11.637	.0880	3.967	.0755	.134	.0556	.017	.0036
W_L x C_1	1	21.132	.0537	8.271	.0347	.002	.8312	.007	.1901
W_L x C_2	1	4.285	.3778	2.961	.1998	.284	.0261	.023	.0165

Table 6--continued.

Source	df[a]	Dry matter intake (kg/d)		Milk yield (kg/d)		Milk fat (%)		Milk fat yield (kg/d)	
		MS[a]	P>F[a]	MS	P>F	MS	P>F	MS	P>F
W_L x C_3	1	16.885	.0834	8.669	.0308	.196	.0626	.030	.0065
W_L x C_4	1	8.362	.2196	2.547	.2339	.096	.1889	<.001	.9148
W_Q x C_1	1	.550	.7512	3.111	.1890	.277	.0280	.039	.0022
W_Q x C_2	1	20.892	.0550	1.915	.3011	.001	.8848	.001	.5406
W_Q x C_3	1	12.559	.1339	4.291	.1241	.086	.2133	.017	.0366
W_Q x C_4	1	8.555	.2144	.730	.5217	.197	.0622	.023	.0151
Ca x R	2	16.710	.0540	1.208	.5068	.171	.0504	.003	.4710
Ca x C_1	1	2.694	.4837	.486	.6011	.043	.3791	.004	.2769
Ca x C_2	1	30.696	.0211	1.928	.2995	.301	.0223	.001	.5729
Ca x C_3	1	15.758	.0941	.726	.5230	.334	.0164	.004	.3028
Ca x C_4	1	17.553	.0777	1.684	.3319	.009	.6848	.002	.5059
W x Ca x R	4	2.841	.7180	1.504	.4958	.026	.7518	.002	.6490
Residual	50	5.411		1.754		.054		.004	

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[a] df = Degrees of freedom; MS = Mean square; P>F = Significance level.

[b] C_1 through C_4 = orthogonal contrasts comparing corn silage versus alfalfa haylage and cottonseed hulls (C_1); alfalfa haylage versus cottonseed hulls (C_2); cottonseed hulls versus alfalfa haylage and corn silage (C_3); alfalfa haylage versus corn silage (C_4); contrasts involving C_1 and C_2 were made in one analysis and those involving C_3 and C_4 were made in a separate analysis.

Table 7. Least-Squares Analysis of Variance of 4% Fat-Corrected Milk (FCM) Yield, Milk Protein (MP) % and Yield, and Body Weight Change (BWC)

Source	df[a]	FCM (kg/d)		MP (%)		MP yield (kg/d)		BWC (kg/35 d)	
		MS[a]	P>F[a]	MS	P>F	MS	P>F	MS	P>F
Cow	35	25.824	.0001	.204	.0001	.037	.0001	228.855	.9315
Period	2	19.372	.0001	.040	.2205	.038	.0001	1942.267	.0085
Whole cottonseed (W)	2	33.777	.0001	.355	.0001	.046	.0001	692.856	.1644
Linear (W_L)	1	44.839	.0001	.656	.0001	.086	.0001	1239.248	.0732
Quadratic (W_Q)	1	23.726	.0005	.049	.1756	.008	.1328	160.440	.5133
Calcium (Ca)	1	.005	.9585	.124	.0334	.006	.1940	53.143	.7063
Roughage (R)	2	.310	.8329	.189	.0017	.011	.0355	3620.188	.0003
C_1[b]	1	.102	.8071	.374	.0004	.016	.0289	120.333	.5711
C_2[b]	1	.519	.5818	.004	.7113	.007	.1564	7123.139	.0001
C_3[b]	1	.613	.5496	.128	.0314	<.001	.9022	6169.868	.0002
C_4[b]	1	.007	.9483	.250	.0032	.023	.0102	1075.459	.0945
W x Ca	2	.392	.7935	.028	.3514	<.001	.9186	299.700	.4507
W_L x Ca	1	.246	.7043	<.001	.9766	<.001	.9958	136.463	.5465
W_Q x Ca	1	.549	.5709	.055	.1504	.001	.6819	455.506	.2726
W x R	4	6.951	.0058	.021	.5144	.008	.0630	685.625	.1335
W_L x C_1	1	5.582	.0750	.027	.3129	.013	.0458	1228.324	.0745
W_L x C_2	1	8.712	.0274	.040	.2229	.007	.1390	64.055	.6792

Table 7--continued.

Source	df[a]	FCM (kg/d)		MP (%)		MP yield (kg/d)		BWC (kg/35 d)	
		MS[a]	P>F[a]	MS	P>F	MS	P>F	MS	P>F
W_L x C_3	1	14.199	.0055	.027	.3129	.018	.0233	109.397	.5891
W_L x C_4	1	.292	.6794	.040	.2229	.003	.3234	1153.811	.0836
W_Q x C_1	1	13.315	.0071	.003	.7436	.007	.1516	593.103	.2114
W_Q x C_2	1	.001	.9771	.017	.4164	.004	.2679	929.649	.1193
W_Q x C_3	1	7.774	.0368	.003	.7436	.009	.0971	1470.874	.0517
W_Q x C_4	1	6.960	.0476	.017	.4164	.002	.4769	37.638	.7511
Ca x R	2	.822	.6172	.130	.0104	.002	.5003	54.721	.8629
Ca x C_1	1	1.643	.3286	.025	.3270	.004	.2851	7.613	.8865
Ca x C_2	1	.001	.9771	.236	.0041	<.001	.6274	101.740	.6024
Ca x C_3	1	.371	.6411	.025	.3270	.003	.3400	53.900	.7044
Ca x C_4	1	1.278	.3888	.236	.0041	.002	.4914	55.188	.7010
Residual	50	1.688		.026		.003		370.098	

[a] df = Degrees of freedom; MS = Mean square; P>F = Significance level.

[b] C_1 through C_4 = orthogonal contrasts comparing corn silage versus alfalfa haylage and cottonseed hulls (C_1); alfalfa haylage versus cottonseed hulls (C_2); cottonseed hulls versus alfalfa haylage and corn silage (C_3); alfalfa haylage versus corn silage (C_4); contrasts involving C_1 and C_2 were made in one analysis and those involving C_3 and C_4 were made in a separate analysis.

Table 8. Effects of Levels of Dietary Whole Cottonseed, Added Limestone and Sources of Roughage on Feed Intake, Milk Yield and Composition, and Body Weight Change

Response	Dietary main effects							
	Levels of WCS (%)[a]			Levels of calcium (%)		Roughage sources[a]		
	0	15	30	.69	1.29	CS	AH	CSH
Dry matter intake, kg/d	24.2[b]	23.0	20.5	22.7	22.4	20.8	23.1	23.7
Milk yield, kg/d	22.3	22.8	21.4	22.1	22.2	22.0	21.9	22.6
FCM,[c] kg/d	20.3	20.5	18.6	19.7	19.8	19.7	19.7	19.9
Milk fat, %	3.46	3.37	3.19	3.35	3.33	3.34	3.31	3.27
Milk fat, kg/d	.76	.76	.67	.73	.72	.73	.73	.72
Milk protein, %	3.52	3.37	3.31	3.44	3.36	3.48	3.37	3.35
Milk protein, kg/d	.78	.76	.70	.75	.74	.76	.73	.74
Body weight change, kg/35 d	22.7	21.0	14.1	30.1	27.8	17.8	9.8	30.3

[a] CS = Corn silage; AH = Alfalfa haylage; CSH = Cottonseed hulls; WCS = Whole cottonseed.

[b] Values are least-squares means.

[c] FCM = 4% Fat-corrected milk.

Increasing WCS in the diet (pooled across roughage sources and dietary calcium contents) decreased dry matter intake in a linear fashion ($P < .0001$). This agrees with the general trend reported for diets when supplemented with fat (Palmquist and Conrad, 1980) and with WCS (Coppock et al., 1985a; Hawkins et al., 1985). There was a 13.7% lower dry matter intake with 30% WCS than in diets without WCS. Calculated NE_1 consumptions were similar (36.3, 36.8 and 34.8 Mcal/kg) among the 0, 15 and 30% WCS-containing diets. Smith et al. (1981) reported no depression in calculated NE_1 intake when WCS was 25 and 30% of diet. They attributed the lack of difference to the fact that all diets were already high in NE_1. Coppock et al. (1985b) reported a 10.7% depression in dry matter intake of CS-based diets as WCS was increased from 0 to 30%, but intakes of NE_1 (calculated) were similar. Hawkins et al. (1985), likewise, reported an 18% reduction in intake with diet based on CS and 18% WCS compared with a control diet containing no WCS. Total NE_1 intakes with both diets were equal, which indicated that cows compensated for the lower energy content of diets containing no WCS by consuming more dry matter than with diets containing WCS. In our study, WCS dry matter consumption averaged 3.5 and 6.1 kg daily for the 15 and 30% WCS additions, or .6 and 1.07% of body weight. This was comparable with the maximum daily intake of WCS (1.05% of body weight) by a Holstein cow reported in Texas (Coppock et al., 1985b).

There was a quadratic response on actual milk yield ($P<.0015$) with increasing level of WCS in the diet (tables 6 and 8). Actual milk yield was highest with 15% WCS but declined by about 6% when dietary WCS was increased from 15 to 30%. Milk fat percent also dropped linearly (P<.0001) with increased WCS inclusion in the diet. This will be discussed later. Yield of 4% fat-corrected milk (FCM) showed a quadratic response to increased WCS addition (table 7 and 8, P<.0005). Reduced yields of both actual milk and FCM with the high WCS-containing diets were rather surprising. Smith (1983) reported similar milk yields of cows fed high WCS (15 and 30% of diet dry matter) compared with cows consuming no WCS. Their diets were based on alfalfa hay which constituted about 50% of the diet dry matter. Except for a higher CP in their diets, concentrations of other nutrients were comparable to those in the present study. In spite of the lower dry matter intake (19 kg/d) compared with 22.5 kg/d in the present study, the early lactation cows in their experiment yielded almost 10 kg more milk. Thus, it is likely also that factors other than diet were responsible for the differences in response, such as stage of lactation and genetic potential for milk production.

Several studies reported increased milk fat percent from WCS feeding (Anderson et al., 1979; Smith and Collar, 1980; dePeters et al., 1985). Results of our experiment (tables 6 and 8) instead indicated a linear decrease in milk fat percent with increasing dietary WCS (P<.0001). Whole cottonseed inclusion at 30% of diet dry matter caused fat percent to drop .27 percentage units compared with no WCS. Yield of milk fat was reduced about 12% with 30% WCS in the diet compared with 0 or 15% WCS. Depression in milk fat percent and yield due to WCS supports results of Van Horn et al. (1984) who fed

12.5% WCS and found a drop in fat percent and yield. Present levels of WCS (15 and 30% of diet dry matter) supplied 2.85 and 5.70% total dietary fat, respectively. Whole cottonseed used in our study contained 19% ether extract.

Effects on percent and yield of milk protein (table 7) similarly showed linear decreases ($P<.0001$) with increasing levels of dietary WCS. Similar effects on milk protein content and yields have been reported with WCS feeding (Anderson et al., 1979; dePeters et al., 1985) and other added dietary fats (de Visser et al., 1982). In spite of a difference in body weight change of 8.6 kg for cows fed 30 and 0% WCS, there was no significant effect of level of WCS on 35-d body weight change. This may be due to the large unaccounted for variation (residual mean squares = 370.098).

Effects of sources of roughages. Source of roughage affected dry matter intake ($P<.0001$, tables 6 and 8). Dry matter intake was highest with CSH-containing diets, and 12 and 3% lower with CS- and AH-containing diets ($P<.0007$). Pooled across all levels of WCS, dry matter intake was lowest with CS-based diets ($P<.0001$). One possible reason for this was the higher NE_1 content (almost 15% higher) of CS-based diets (1.77 Mcal/kg) compared with AH- and CSH-based diets (table 4). Thus, cows may not have required as much CS-based diet dry matter to meet their NE_1 requirement. Dry matter intake of CS-based diet was about 12.5% lower than diets based on AH and CSH, yet calculated NE_1 intakes were similar (37.4, 37.0 and 35.5 for CS-, AH- and CSH-based diets, respectively). Higher dry matter intakes of

CSH-based diets compared with diets based on other roughage sources were reported earlier (McCoy et al., 1965; Villaviciencio et al., 1968; Harris et al., 1983; Van Horn et al., 1984).

Harris et al. (1983) reported about 25% higher dry matter intake with a CSH-based diet (CSH included at 30 to 40% of diet dry matter) compared with a diet containing CS. Van Horn et al. (1984) compared CSH (30 to 35% of dry matter) with corrugated cardboard boxes and a combination of cardboard boxes and peanut hulls as alternative fiber sources. They reported 16 and 30% higher intakes with the CSH-containing diet than diets containing corrugated boxes and a combination of corrugated boxes and peanut hulls. In the present experiment, roughage source affected actual milk yield (P<.0629) but not FCM yield (P>.8329) (table 8). Actual milk yields were similar between CS- and AH-based diets, but were lower than with CSH-based diets (P<.0226). However, when adjusted to equal dry matter intake [dry matter intake in the model as a continuous independent variable (covariate)], the effect of roughages on actual milk yield was no longer detected (P > .150). McCoy et al. (1965) and Harris et al. (1983) made similar observations that milk yield was higher with CSH-based diets than those with CS. The higher milk yield probably resulted from higher dry matter intake. Villaviciencio et al. (1968) reported no effect of roughage source (CSH versus CS in complete rations) on milk yield, whereas others reported higher actual milk yields from CSH- compared with AH-containing diets (Sargent et al., 1975; Van Horn et al., 1979).

Dietary roughage source also affected milk fat percent (P<.0505). Corn silage- and AH-based diets produced milk with the highest milk fat percent. Milk fat percent of CSH-based diet was lower (P<.0336) than CS- or AH-based diets. Diets based on CS was superior (P<.0004) to those containing AH and CSH in increasing average milk protein percent (table 8). Milk fat yield also was higher with CS- than CSH- or AH-based diets (P<.0289). There was an effect of roughage source on change in body weight (P<.0003, table 7). Cows receiving CSH-based diets had the highest average body weight gain (30.3 kg/35 d) compared with 17.8 and 9.8 kg/35 d with CS- and AH-based diets (P<.0002, table 8). Average 35-d weight gains on CS-based diets were larger (P<.0945) than gain by AH-based diets. Higher 35-d weight gains with CS-based diets possibly were attributed to the higher energy content of these diets compared with AH- and CSH-containing diets. Higher body weight gains with CSH-based diets could be due to higher dry matter intake, therefore resulting in greater gut-fill. When weight gain data were adjusted to equal feed intake (by including dry matter intake as a continuous independent variable in the model) differences in weight gains between cows receiving CS- and CSH-based diets diminished (P>.1500). Morales (1986) also reported higher weight gains of cows receiving CSH-containing diets than those receiving AH-based diets.

Interactions of whole cottonseed addition and roughage source. Figures 1 through 6 illustrate the interactions of WCS level with the roughage source. There was an interaction (P<.0880) of WCS level with rougage source on daily dry matter intake (figure 1). Without WCS addition, dry matter intake of CS-based diets was lowest compared with diets based on AH or CSH. Dry matter intake of CSH- and AH-based diets declined more rapidly as WCS addition was increased to 15 and 30% of the diet dry matter compared with the CSH-based diet. Effect was markedly severe with the CSH-based diets at 30% WCS but not with 15% WCS. Interactive effect of WCS level and roughage source on actual milk yield is in figure 2 (P<.0755). Overall, actual milk yield declined with CSH- and AH-based diets as WCS level increased from 0 to 30%. However, yield with 15% WCS was higher than with 0 or 30% WCS in CSH- or AH-based diets. Biggest decline was with CSH-based diet. Similar relationships were noted for FCM yield (figure 3) and milk fat percent and yield (figures 4 and 5). Among interactions of WCS level with roughage on milk protein yield also was detected (P=.0630, figure 6). Much of this effect was attributed to differences in response to the WCS level (at 15 and 30% inclusion).

An interaction between WCS and roughage source was suggested by Van Horn et al. (1984) for both milk yield and feed intake. They reported greater responses when WCS was added to diet deficient in effective fiber. With pelleted steam pressure-treated bagasse, milk yield was increased more compared with CS- or CSH-containing diets. In our study, 15% WCS addition appeared most effective for increasing FCM yield of CSH- and AH-containing diets. With 30% WCS, milk yield declined with the AH- and CSH-based diets. Only with CS-based diets

Figure 1. Interaction between level of whole cottonseed and roughage source ($P<.0880$) on dry matter intake. (AH = alfalfa haylage; CS = corn silage; CSH = cottonseed hulls)

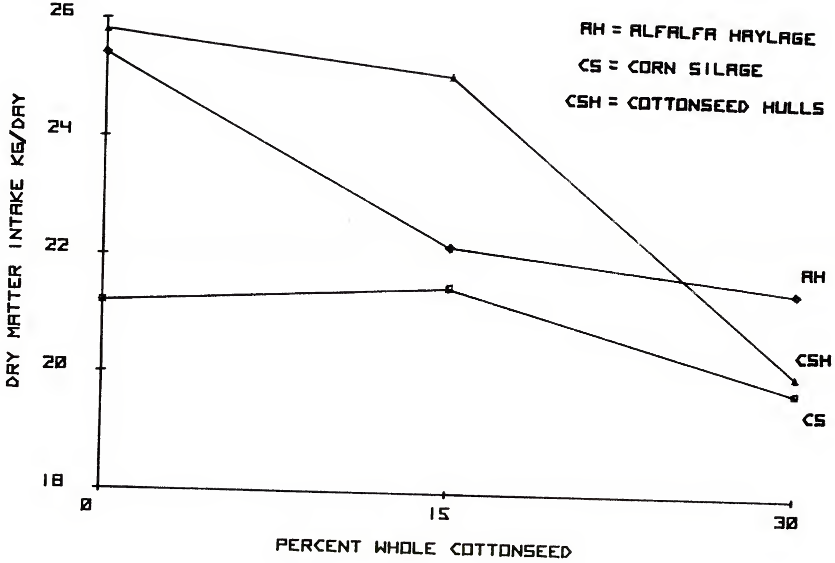

AH = ALFALFA HAYLAGE
CS = CORN SILAGE
CSH = COTTONSEED HULLS
DRY MATTER INTAKE KG/DAY
26
24
22
20
18
0
15
30
PERCENT WHOLE COTTONSEED
AH
CSH
CS

Figure 2. Interaction between level of whole cottonseed and roughage source ($P<.0755$) on milk yield. (AH = alfalfa haylage; CS = corn silage; CSH = cottonseed hulls)

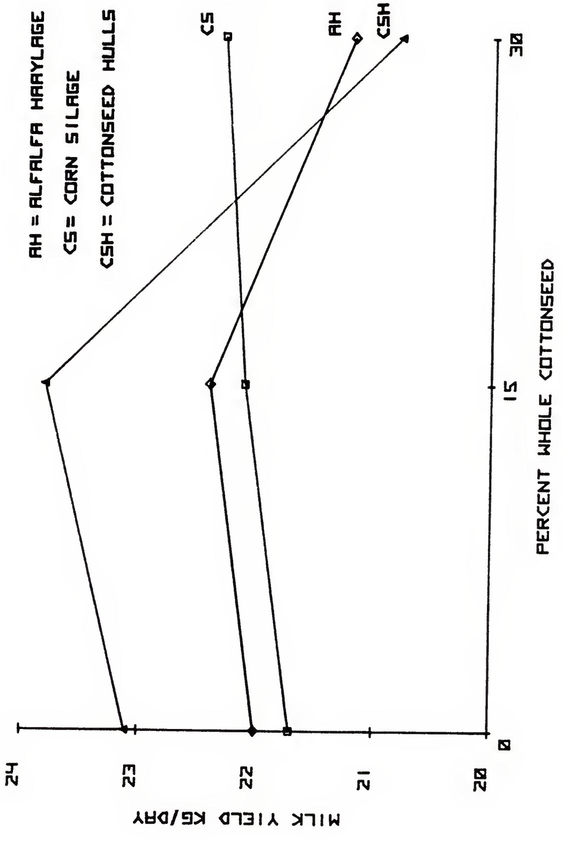

AH = ALFALFA HAAYLAGE
CS= CORN SILAGE
CSH = COTTONSEED HULLS
CS
AH
CSH
MILK YIELD KG/DAY
24
23
22
21
20
0
15
30
PERCENT WHOLE COTTONSEED

Figure 3. Interaction between level of whole cottonseed and roughage source ($P<.0058$) on 4% fat-corrected milk yield. (AH = alfalfa haylage; CS = corn silage; CSH = cottonseed hulls)

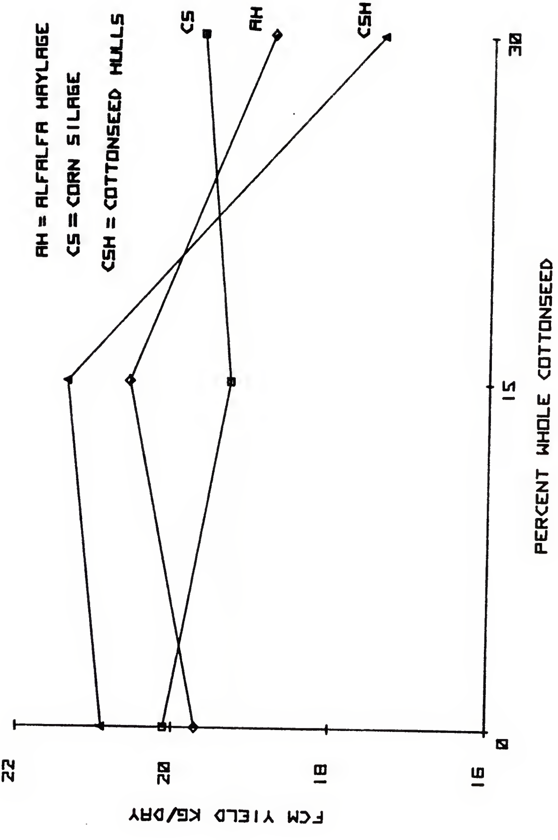

AH = ALFALFA HAYLAGE
CS = CORN SILAGE
CSH = COTTONSEED HULLS
CS
AH
CSH
FCM YIELD KG/DAY
22
20
18
16
0
15
30
PERCENT WHOLE COTTONSEED

Figure 4. Interaction between level of whole cottonseed and roughage source ($P<.0556$) on milk fat percent. (AH = alfalfa haylage; CS = corn silage; CSH = cottonseed hulls)

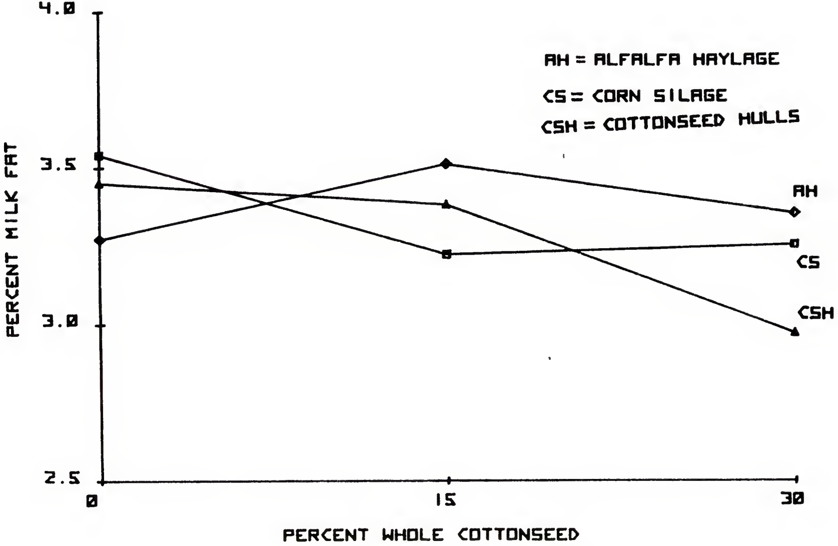

AH = ALFALFA HAYLAGE
CS = CORN SILAGE
CSH = COTTONSEED HULLS
PERCENT MILK FAT
4.0
3.5
3.0
2.5
0
15
30
PERCENT WHOLE COTTONSEED
AH
CS
CSH

Figure 5. Interaction between level of whole cottonseed and roughage source ($P<.0036$) on milk fat yield. (AH = alfalfa haylage; CS = corn silage; CSH = cottonseed hulls)

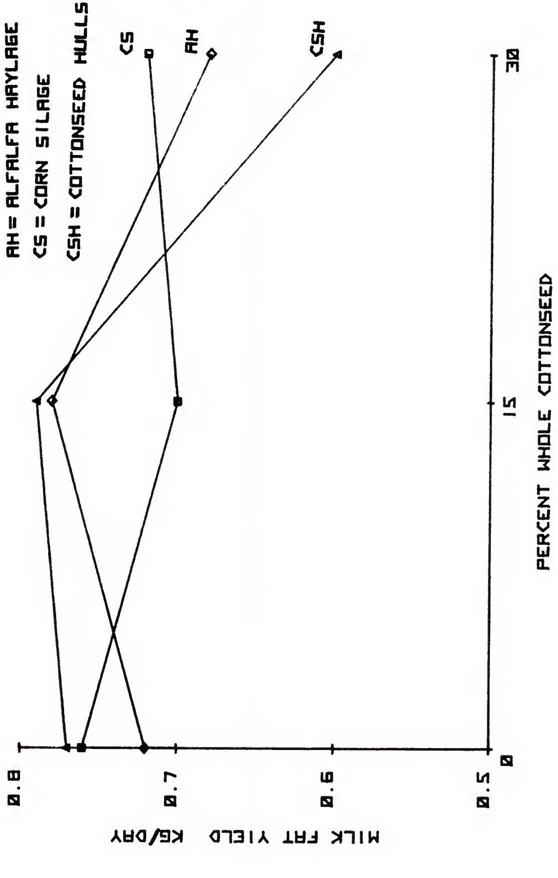
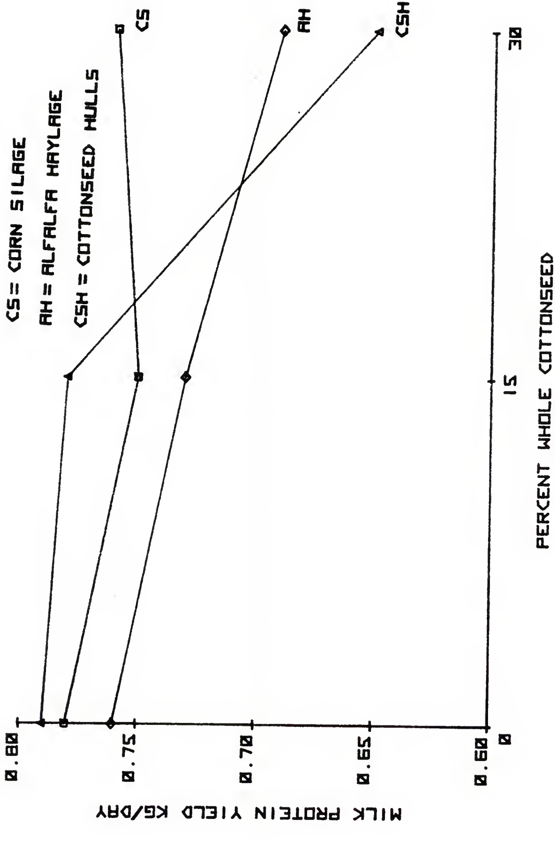

AH = ALFALFA HAYLAGE
CS = CORN SILAGE
CSH = COTTONSEED HULLS
CS
AH
CSH
MILK FAT YIELD KG/DAY
0.8
0.7
0.6
0.5
0
15
30
PERCENT WHOLE COTTONSEED

Figure 6. Interaction between level of whole cottonseed and roughage source ($P<.0630$) on milk protein yield. (AH = alfalfa haylage; CS = corn silage; CSH = cottonseed hulls)


CS = CORN SILAGE
AH = ALFALFA HAYLAGE
CSH = COTTONSEED HULLS
CS
AH
CSH
MILK PROTEIN YIELD KG/DAY
0.80
0.75
0.70
0.65
0.60
0
15
30
PERCENT WHOLE COTTONSEED

was actual milk yield increased with 30% WCS addition compared with 15 or 0% WCS.

There were no main effects of dietary calcium on any lactational performance estimates except milk protein percent ($P<.0334$). Milk protein percent was decreased slightly with higher dietary calcium (table 8). There was a calcium level by roughage source interaction on dry matter intake ($P<.0540$, figure 7). Dry matter intake responded differently to added calcium in AH- compared with CSH-based diets ($P<.0211$). At the lower level of dietary calcium, intake was highest with the CSH-based diet, whereas 1.29% calcium in the diet depressed intake of CSH- and CS-based diets compared with .69% calcium. However, intake of AH-based diets was improved by 9% with the higher level of dietary calcium.

Interactive effect of dietary calcium and roughage source on milk fat content indicated that with .69% calcium, highest milk fat percent (3.49%) was with AH-based diets and lowest (3.17%) with CSH-based diets ($P<.0504$, figure 8). However, with 1.29% calcium, milk fat percent increased .2 percentage units with CSH-based diets but declined with CS- and AH-based diets compared with .69% calcium in the diet. Figure 9 illustrates interaction of calcium level with dietary roughage source on milk protein percent ($P<.0104$). The higher level of calcium (1.29% of diet dry matter) increased milk protein percent over .69% calcium in the CSH-containing diets. Corn silage- and AH-based diets both had lower milk protein percents at 1.29% calcium than at .69% calcium.

Figure 7. Interaction between level of calcium and roughage source ($P<.0540$) on dry matter intake. (AH = alfalfa haylage; CS = corn silage; CSH = cottonseed hulls)

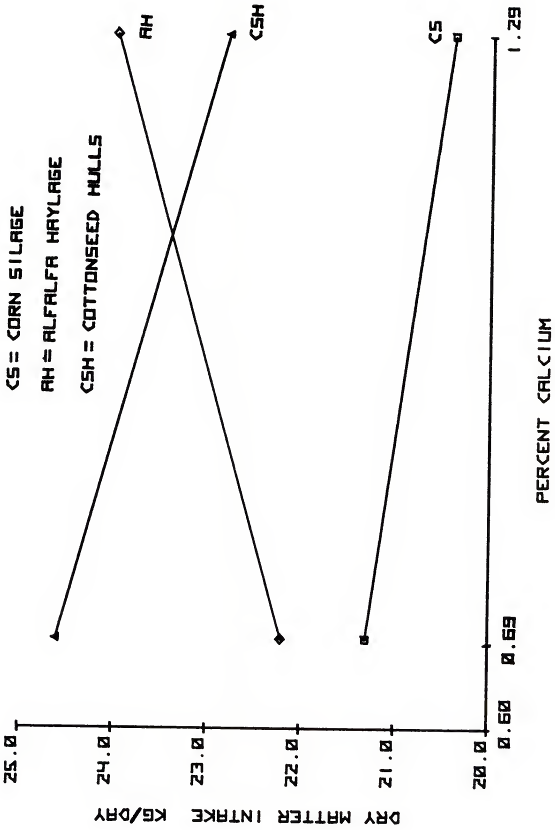

CS = CORN SILAGE
AH = ALFALFA HAYLAGE
CSH = COTTONSEED HULLS
AH
CSH
CS
DRY MATTER INTAKE KG/DAY
25.0
24.0
23.0
22.0
21.0
20.0
0.60
0.69
1.29
PERCENT CALCIUM

Figure 8. Interaction between level of calcium and roughage source ($P<.0504$) on milk fat percent. (AH = alfalfa haylage; CS = corn silage; CSH = cottonseed hulls)

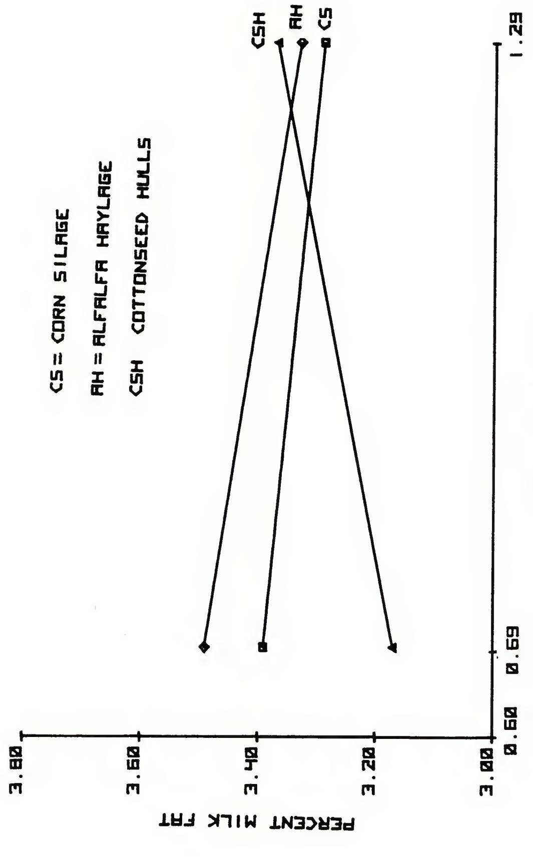
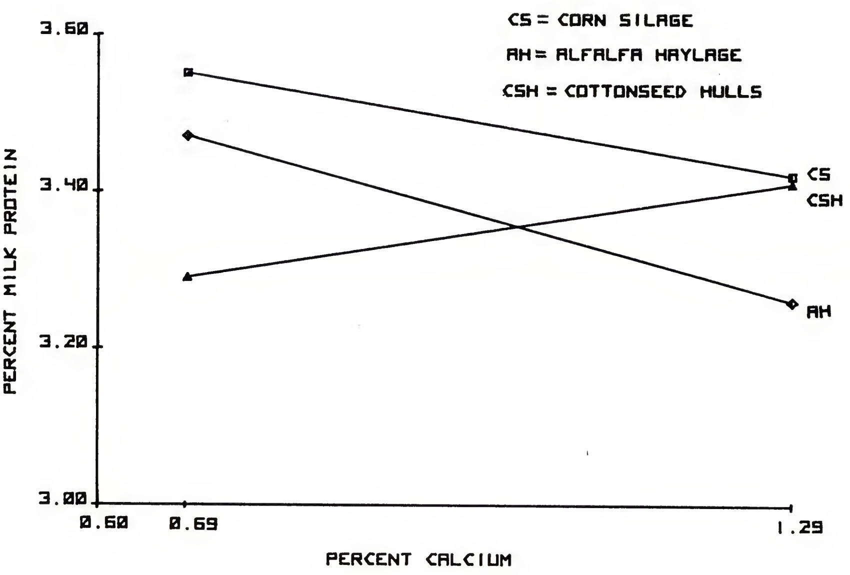

CS = CORN SILAGE
AH = ALFALFA HAYLAGE
CSH COTTONSEED HULLS
CSH
AH
CS
PERCENT MILK FAT
3.00
3.20
3.40
3.60
3.80
PERCENT CALCIUM
0.60
0.69
1.29

Figure 9. Interaction between level of calcium and roughage source ($P<.0104$) on milk protein percent. (AH = alfalfa haylage; CS = corn silage; CSH = cottonseed hulls)


CS = CORN SILAGE
AH = ALFALFA HAYLAGE
CSH = COTTONSEED HULLS
CS
CSH
AH
3.60
3.40
3.20
3.00
0.60
0.69
1.29
PERCENT MILK PROTEIN
PERCENT CALCIUM

Availability of calcium and phosphorus of organic sources are lower than inorganic sources due to interference by phytate and oxalic acids (Maynard et al., 1979). This suggests that calcium supplied by AH (about .4% of total diet dry matter) was not readily available. At the lower calcium level (.69%), a large proportion (58%) of total dietary calcium in the AH-based diets were from AH, whereas at the higher calcium level, the proportion of total dietary calcium contributed by AH was much smaller (31%). It is not clear how this stimulated intake of AH-based diet.

Digestibilities of Dry Matter, Neutral Detergent Fiber, Acid Detergent Fiber and Cellulose

Apparent digestion coefficients in this experiment were estimated using permanganate lignin as an internal digestibility marker. Effects of dietary treatment factors on apparent digestion coefficients of dry matter and fiber components are summarized in tables 9 and 10. Overall, mean apparent digestion coefficients for dry matter, NDF, ADF and cellulose were 70.1, 60.7, 54.2 and 62.8%, respectively. These values were in close agreement with results reported by Bernard and Amos (1985). They used acid insoluble ash (AIA) as internal marker for comparing effects due to inclusion of a fixed quantity (2.27 kg/d) of WCS (loose versus pelleted) with the mean dry matter intake at 21.45 kg; this amounted to 10.5% WCS. Their estimate of apparent digestion coefficients of dry matter, NDF, ADF and cellulose were 71.7, 60.5, 58.7 and 64.6% with diets containing loose WCS.

Fiber digestion as reflected by the apparent digestion coefficients of DM, NDF, ADF and cellulose in our study were enhanced

Table 9. Least-Squares Analysis of Variance for Coefficients of Apparent Digestibility of Dry Matter (DM), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Cellulose

Source	df[a]	DM		NDF		ADF		Cellulose	
		MS[a]	P>F[a]	MS	P>F.	MS	P>F	MS	P>F
Cow	35	175.567	.6244	301.025	.6739	441.130	.6852	363.607	.6853
Period	2	858.342	.0174	752.544	.1264	1671.390	.0477	1144.537	.0778
Whole cottonseed (W)	2	264.908	.2666	1384.520	.0252	2917.535	.0061	961.544	.1151
Linear (W_L)	1	199.591	.3167	2260.702	.0141	4736.360	.0039	1601.891	.0581
Quadratic (W_Q)	1	318.771	.2071	461.640	.2555	999.296	.1704	290.005	.4131
Calcium (Ca)	1	2.359	.9129	0.012	.9954	282.548	.4630	30.247	.7909
Roughage (R)	2	629.951	.0480	522.644	.2334	823.967	.2130	355.304	.4401
C_1[b]	1	64.323	.5684	7.983	.8804	735.238	.2385	180.192	.5183
C_2[b]	1	1206.396	.0163	1040.583	.0904	945.962	.1821	542.853	.2642
C_3[b]	1	701.177	.0638	723.350	.1562	181.742	.5557	189.254	.5080
C_4[b]	1	589.979	.0882	346.215	.3240	1491.097	.0955	536.788	.2669
W x Ca	2	158.242	.4502	265.208	.4730	171.512	.7190	321.611	.4752
W_L x Ca	1	75.739	.5361	260.608	.3916	87.856	.6818	315.784	.3933
W_Q x Ca	1	244.166	.2686	276.578	.3776	258.964	.4822	335.648	.3789
W x R	4	83.537	.7875	201.631	.6800	194.426	.8244	177.255	.7961
W_L x C_1	1	2.402	.9121	9.354	.8706	7.341	.9056	10.200	.8776

Table 9--continued.

Source	df[a]	DM MS[a]	DM P>F[a]	NDF MS	NDF P>F	ADF MS	ADF P>F	Cellulose MS	Cellulose P>F
W_L x C_2	1	249.123	.2639	520.815	.2276	125.266	.6246	308.973	.3984
W_L x C_3	1	211.063	.3033	458.440	.2572	70.791	.7128	285.846	.4165
W_L x C_4	1	43.889	.6374	78.777	.6368	63.581	.7272	37.397	.7682
W_Q x C_1	1	84.927	.5124	266.830	.3861	35.531	.4100	291.267	.4121
W_Q x C_2	1	11.171	.8119	43.996	.7240	297.400	.4515	123.640	.5924
W_Q x C_3	1	56.844	.5918	195.404	.4578	598.605	.2869	332.645	.3810
W_Q x C_4	1	39.804	.6535	117.314	.5646	60.290	.7341	85.310	.6564
Ca x R	2	590.252	.0575	995.158	.0672	1690.696	.0461	1185.083	.0714
Ca x C_1	1	1164.280	.0182	1959.475	.0217	3191.764	.0163	2361.677	.0225
Ca x C_2	1	11.167	.8119	21.746	.8039	159.590	.5808	3.751	.9256
Ca x C_3	1	415.370	.1508	714.227	.1588	1593.304	.0852	706.139	.2038
Ca x C_4	1	773.655	.0519	1290.523	.0602	1813.652	.0668	1680.395	.0525
W x Ca x R	4	270.852	.2515	472.037	.2673	1262.751	.0585	633.681	.2199
Residual	50	195.115		348.957		516.523		425.811	

[a] df = Degrees of freedom; MS = Mean square; P>F = Significance level.

[b] C_1 through C_4 = orthogonal contrasts comparing corn silage versus alfalfa haylage and cottonseed hulls (C_1); alfalfa haylage versus cottonseed hulls (C_2); cottonseed hulls versus alfalfa haylage and corn silage (C_3); alfalfa haylage versus corn silage (C_4); contrast involving C_1 and C_2 were made in one analysis and those involving C_3 and C_4 were made in a separate analysis.

Table 10. Effects of Levels of Dietary Whole Cottonseed, Added Limestone and Sources of Roughage on Coefficients of Apparent Digestibility of Dry Matter, Neutral Detergent Fiber, Acid Detergent Fiber and Cellulose

Response	Dietary main effects							
	Levels of WCS (%)[a]			Levels of calcium (%)		Roughage sources[a]		
	0	15	30	.69	1.29	CS	AH	CSH
Dry matter digestibility	67.1[b]	72.6	70.5	70.2	69.9	71.2	65.2	73.8
Neutral detergent fiber digestibility	53.4	63.8	65.0	60.7	60.7	61.1	56.6	64.5
Acid detergent fiber digestibility	43.3	58.7	60.4	52.3	56.0	58.0	48.5	56.1
Cellulose Digestibility	56.6	65.1	66.4	62.2	63.3	64.6	58.9	64.7

[a] CS = Corn silage; AH = Alfalfa haylage; CSH = Cottonseed hulls; WCS = Whole cottonseed.

[b] Least-squares means.

by the addition of WCS to the diet. Roughage source affected dry matter digestibility ($P<.0480$) but not digestibility of dietary fiber components. Dry matter digestibility was highest with CSH-containing (73.8%) and lower with AH- (65.2%) and CS- (71.2%) containing diets ($P<.0638$). Effects of WCS level on NDF digestibility ($P=.0252$) and ADF digestibility ($P=.0061$) were detected. Both NDF ($P<.0141$) and ADF ($P<.0039$) digestibilities improved linearly with increasing dietary levels of WCS. Dry matter digestibility was not affected by WCS level ($P>.2666$).

There were significant interactions between calcium and roughage source on dry matter ($P<.0575$), NDF ($P<.0672$), ADF ($P<.0461$) and cellulose ($P<.0714$) digestibilities. Interactions between calcium and roughage source on digestibility of dietary fiber components (NDF and ADF) are in figures 10 and 11. At the lower calcium level (.69% of the diet dry matter), apparent digestion coefficient for CS-based diets was higher than for CSH- or AH-based diets. Additional calcium (1.29%) depressed detergent fiber digestibilities of CS-based diets but enhanced those of CSH- and AH-based diets (calcium level by roughage source interaction, $P<.0217$ for NDF digestibility and $P<.0163$ for ADF digestibility).

Figure 10. Interaction between level of calcium and roughage source ($P<.0672$) on neutral detergent fiber (NDF) digestibility. (AH = alfalfa haylage; CS = corn silage; CSH = cottonseed hulls)

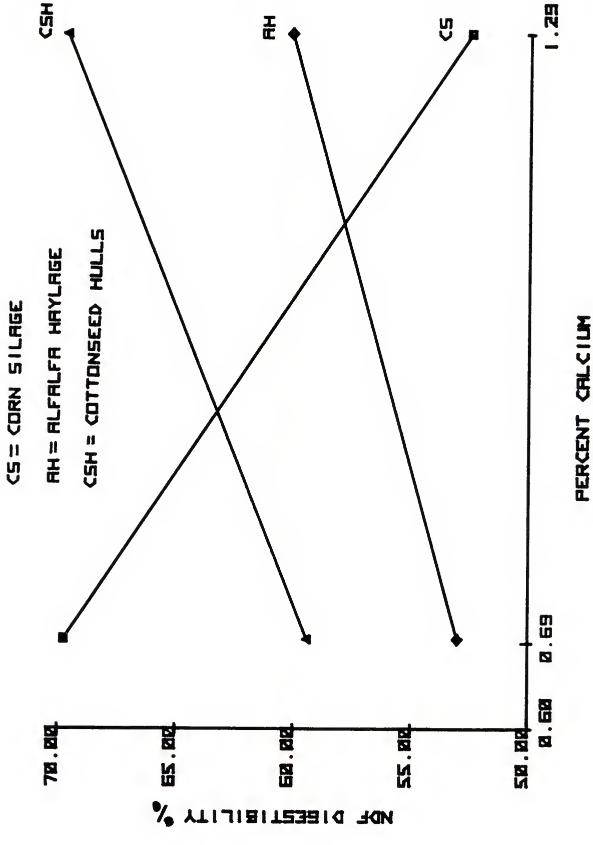
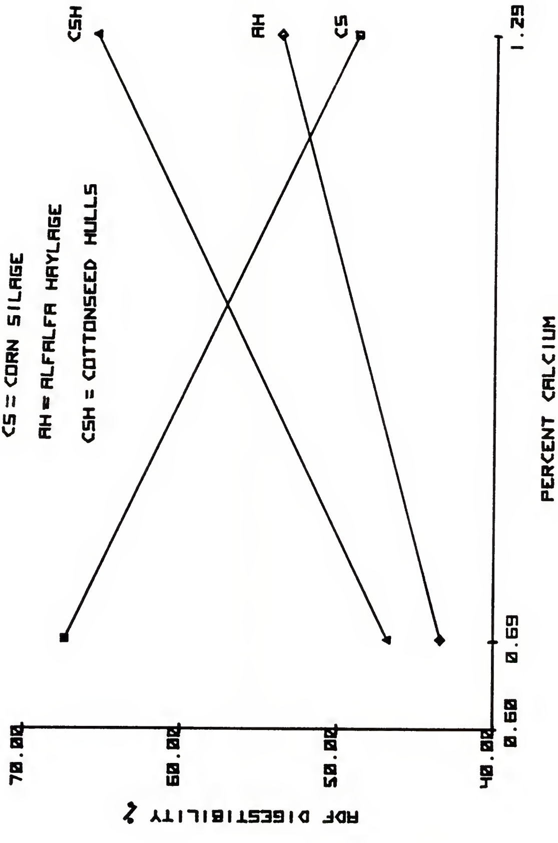

CS = CORN SILAGE
AH = ALFALFA HAYLAGE
CSH = COTTONSEED HULLS
CSH
AH
CS
NDF DIGESTIBILITY %
70.00
65.00
60.00
55.00
50.00
0.60
0.69
1.29
PERCENT CALCIUM

Figure 11. Interaction between level of calcium and roughage source ($P<.0461$) on acid detergent fiber (ADF) digestibility. (AH = alfalfa haylage; CS = corn silage; CSH = cottonseed hulls)


CS = CORN SILAGE
AH = ALFALFA HAYLAGE
CSH = COTTONSEED HULLS
CSH
AH
CS
ADF DIGESTIBILITY %
70.00
60.00
50.00
40.00
0.60
0.69
1.29
PERCENT CALCIUM

CHAPTER IV

EFFECTS OF CALCIUM HYDROXIDE TREATMENT OF WHOLE COTTONSEED AND SOURCES OF SUPPLEMENTAL PROTEIN ON DRY MATTER INTAKE AND APPARENT DIGESTIBILITIES OF DRY MATTER, ACID DETERGENT FIBER AND CRUDE PROTEIN BY NONLACTATING HOLSTEIN COWS

Introduction

One of the biggest potential problems of feeding oilseeds such as whole cottonseed to ruminants is reduced fiber digestion associated with added fat from the seed. Fat supplementation has reduced fiber digestibility (Czerkawski et al., 1966; Johnson and McClure, 1973) and voluntary feed intake by steers (Johnson and McClure, 1973). Kowalczyk et al. (1977) reported reduced fiber disappearance from dacron bags incubated in the rumen when diets contain supplemental fat.

It was suggested that added calcium in the diet could help minimize inhibitory effects of high dietary fat on fiber digestion. This was evident from studies of Brooks et al. (1954), Johnson and McClure (1973) and Palmquist and Conrad (1980). Brooks et al. (1954) reported that alfalfa ash caused a reversal of the depressing effects of corn oil and lard on fiber digestibility. The effect was speculated to be due to high calcium in alfalfa ash. Results of Johnson and McClure (1973) indicated improved digestibility of fat-supplemented corn silage which had limestone added to it at the time of ensiling. Palmquist and Conrad (1980) suggested that a dietary calcium content of .9 to 1% of total diet dry matter could be effective in correcting digestibility depressions of dietary fiber with added fat. However, results of Spearow and Clarke (1984) using calcium carbonate did not support this suggestion in spite of a high calcium content in the diet (1.03% of dry matter) when whole sunflower seed was added to diet (5.7% total ether extract in the diet).

Mechanism by which calcium aids in preventing depression of fiber digestion by high dietary fat is unclear. Jenkins and Palmquist (1982) postulated that calcium caused fatty acids to precipitate out in the ruminal contents as calcium salts (soaps) of the fatty acids, thereby decreasing the negative effects fatty acids may have on microbial digestion of fiber. They pointed out that intraruminal soaping rarely caused more than 50% of dietary fatty acids to precipitate as calcium-fatty acid soaps and that it took several hours before significant quantities of soaps were formed.

Ruminal soaping also is dependent to a large extent on the level and source of calcium. More readily water-soluble calcium sources are more effective in precipitating fatty acids as exemplified by calcium chloride compared with dicalcium phosphate (Jenkins and Palmquist, 1982). The sources of calcium commonly used in ruminant diets, calcium carbonate (limestone) and dicalcium phosphate would not be expected to be very effective for ruminal soaping. An alternative source would be calcium hydroxide which is moderately soluble in water and could be available from other commercial uses. Further, if sufficient reaction time was allowed for maximum soaping by treating the supplemental fat for some predetermined interval prior to feeding, increased quantities of fatty acids may form soaps.

When feeding diets with high fat contents to cows, it was emphasized that special consideration be given to the amount and quality of protein in the diet (Palmquist, 1983). A high quality supplemental protein with high ruminal escape characteristics perhaps would be of better nutritional value to the cow. Among such feeds are brewer's dried grains and distiller's dried grains each with estimated ruminal undegradable protein values of 48 and 33%, respectively (Krishnamoorthy et al., 1982). Roffler et al. (1984) reported a higher milk fat percent when cows were fed a corn gluten meal-supplemented diet compared with a diet supplemented with soybean meal. Corn gluten meal similarly is not readily degradable in the rumen; however, the basal diet in this study (Roffler et al., 1984) did not contain additional fat.

The following experiment was conducted with focus on the following objectives: to compare effects of diets containing whole cottonseed with or without calcium hydroxide treatment on apparent digestibilities of dry matter, acid detergent fiber and crude protein and on ruminal soaping; to study effects of soybean meal and distiller's dried grains with solubles as supplemental protein sources in diets containing whole cottonseed on dry matter intake, body weight change and apparent digestibility of diet dry matter, acid detergent fiber and crude protein; and to compare effects of these dietary treatment variables on ruminal digestion measurements for 12 h after feeding.

Materials and Methods

Animals and Design

Eight nonlactating Holstein cows were used in two trials involving intact whole cottonseed (WCS) (trial I) or crushed WCS (trial II). In each trial, dietary treatment factors were WCS with or without soaking for 22 h in calcium hydroxide and two sources of supplemental dietary protein (soybean meal=SBM or distiller's dried grains with solubles=DDGS) in a 2x2 factorial arrangement. Cows in trial II were fitted with ruminal cannulae for subsequent studies of ruminal digestion. Experimental design was a partially balanced incomplete block with three periods of 28 d each. In each period, the first 14 d were for adaptation to dietary treatments and the remaining 14 d were for collection. Each cow received a different dietary treatment (table 11) in each period.

Formulation and Preparation of the Diets

Diets were formulated (table 12) to contain 16% crude protein and 70% total digestible nutrients (TDN). Values of TDN for the roughages were estimated from regression equations originally derived from the analyzed chemical composition of similar feedstuff types (New York Dairy Herd Improvement Forage Testing Laboratory, Ithaca, NY). In all dietary treatments, WCS was 20% of the diet dry matter. In odd-numbered diets (trial I) WCS was intact and in even-numbered diets (trial II) WCS was crushed. In diets 3, 4, 7 and 8, DDGS was 28.7% of the dry matter. Crude protein equal to that contributed by DDGS was provided in diets 1, 2, 5 and 6 by adding SBM

Table 11. Dietary Treatment Sequence Assignment

Trial[a]	Cow	Period		
		1	2	3
I	796	1[b]	7	3
	808	3	1	5
	862	7	5	1
	1024	5	3	7
II	655	4	2	6
	744	6	4	8
	824	2	8	4
	1114	8	6	2

[a] Trial I was with intact whole cottonseed; trial II was with crushed whole cottonseed.

[b] Diet number.

Table 12. Formulation of Dietary Treatments

Ingredients	Diet number[a]			
	1 and 2	3 and 4	5 and 6	7 and 8
Whole cottonseed	20.0[b]	20.0	20.0	20.0
Grass hay	25.0	25.0	25.0	25.0
Cottonseed hulls	25.0	25.0	25.0	25.0
Distiller's dried grains plus solubles	-	28.7	-	28.7
Soybean meal	13.0	-	13.0	-
Crimped corn	15.6	-	15.6	-
Limestone	.9	.9	1.45	1.5
Dicalcium phosphate	.2	-	.2	-
Magnesium oxide	.1	-	.1	-
Calcium hydroxide	.4	.4	-	-

[a] Odd-numbered diets (trial I) contained intact whole cottonseed (WCS) and even-numbered diets (trial II) contained crushed WCS.

[b] All values are in percent, dry matter basis.

(13.0%) and crimped corn (15.6%). All diets contained 25% CSH and 25% grass hay, dry basis. Supplemental limestone was used to equalize calcium (Ca) from calcium hydroxide treatment across all diets. All other minerals and vitamins were equal in all diets. Analyzed chemical compositions of the diets and feed ingredients are in table 13. For trial II, crushed WCS was prepared by passing it through a corn crimper twice. Treatment of WCS with calcium hydroxide was done by adding a weighed amount of WCS into a freshly prepared aqueous suspension of calcium hydroxide (2 parts water:1 part calcium hydroxide, liter:kg) in individual 150 liter plastic bags. Amount of calcium hydroxide used was 2% of WCS by weight (dry basis). Water was 55 C at the beginning of mixing. The entire contents were mixed for 5 min to make a homogeneous preparation of WCS and calcium hydroxide suspension. These preparations stood at ambient temperature for 22 h. Grass hay was chopped (6 cm or less) and kept in a separate bin. Cottonseed hulls (CSH) were mixed with the rest of the ingredients in the concentrate mix and bagged separately. Daily rations were prepared for each cow each morning by mixing CSH plus concentrate with grass hay and WCS (with or without calcium hydroxide treatment) in a cement mixer for 2 min.

Feeding and Management

For 30 d prior to beginning period I, cows were fed a standardization diet (diet 1, table 12). Thereafter, they were fed their respective treatment diets twice daily at 0730 and 1930 h, in individual feed bunks. Feeding was *ad libitum* with allowance for between 10 and 20% weighback. Adjustments of the amount fed were

Table 13. Chemical Composition of Diets and Some Feed Ingredients

Item	Dry matter[a]	TDN[b]	Crude protein	ADF[b]	Calcium	Magnesium	Phosphorus
	%						
Diets 1+2[c]	78.1[d]	68.1	16.4	40.1	.78	.32	.37
Diets 3+4	78.9	69.2	15.9	43.8	.71	.28	.39
Diets 5+6	89.3	68.1	16.4	40.1	.73	.32	.37
Diets 7+8	89.8	69.1	15.8	43.7	.72	.29	.39
Soybean meal	90.9	81.0	56.6	8.9	.32	.39	.82
Distiller's dried grains plus solubles	98.0	84.0	29.2	21.6	.08	.29	.75
Whole cottonseed	94.6	93.5	19.6	44.1	.14	.38	.50
Cottonseed hulls	92.7	51.6	5.7	67.2	.12	.19	.10
Grass hay	92.7	54.0	8.5	48.1	.41	.31	.19

[a] As fed basis.

[b] TDN = Total digestible nutrient; ADF = Acid detergent fiber.

[c] Diets 1, 2, 5 and 6 have soybean meal for supplemental protein; 3, 4, 7 and 8 have Distiller's dried grains plus solubles as supplemental protein.

[d] All values calculated in dry matter basis.

made as necessary based on the previous day's weighback. Cows were confined to tie-stalls throughout the experiment except when moved to record body weights at the start of the experiment and at the end of each period. Cows were released into a sand lot on two afternoons just prior to the beginning of periods 2 and 3.

Moisture contents of the complete mixed rations and weighbacks were determined. Feed consumption was recorded daily by taking the difference between the amount offered and the weighback. Dry matter intake was calculated based on the moisture contents of the feed and the weighback. Cows had access continuously to individual water cups.

Digestibility Estimations

Chromic oxide was used as an indigestible marker. Marker was administered orally with a dosing gun to each cow in 5 g (96% dry matter) doses in gelatin capsules twice daily at 0730 an 1930 h on days 15 through 24 of each period. Grab samples of feces were obtained at 12 h intervals beginning at 0730 h on day 20 for 5 consecutive days. Fecal samples were frozen (-10 C). After each collection period, samples were thawed at room temperature and composited within cow-period. After thorough mixing, 250 g subsamples were taken in quadruplicate for dry matter determinations (60 C for 48 h). Dried samples were ground in a Wiley mill (2 mm screen) and retained for subsequent analyses for acid detergent fiber (ADF) (Goering and Van Soest, 1970) and nitrogen content (N) by the macro-Kjeldahl method (AOAC, 1970). From this, crude protein (CP) was calculated by multiplying by 6.25. Fecal chromium concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer 5000, Norwalk, CT) after samples were prepared as described by Williams et al. (1962).

The following equations (Schneider and Flatt, 1975) were used for calculating digestion coefficients:

$$1.\quad \text{\% Dry matter digestibility} = 100 - 100 \times \frac{(\text{\% marker in feed})}{(\text{\% marker in feces})}$$

$$2.\quad \begin{array}{c}\text{\% Digestibility}\\ \text{of ADF and CP}\end{array} = 100 - 100 \times \frac{(\text{\% marker in feed})}{(\text{\% marker in feces})} \times \frac{(\text{\% ADF or CP in feces})}{(\text{\% ADF or CP in feed})}$$

Percent marker in feed was calculated as the amount of chromic oxide dosed daily divided by the amount of feed dry matter consumed daily.

Rumen Sampling and Analyses

On the last day of each period (day 28), samples of ruminal liquor were obtained from each cow via ruminal fistula. Using a suction strainer, about 250 ml samples were collected hourly into a plastic bottle for 12 h consecutively beginning at 0715 h (prefeeding). Measurement for ruminal liquor pH was done immediately following collection.

For analysis of volatile fatty acids (VFA) in ruminal liquor, 30 ml aliquots were placed in screw-capped plastic bottles containing 5 g metaphosphoric acid to arrest fermentation and begin protein precipitation. Bottles were shaken vigorously to mix samples with acid, and samples were frozen (-10 C). Final preparation of samples involved centrifugation at 10,000 x g for 20 min; the supernatant was filtered through a disc into a 2 ml sample vial. Volatile fatty acid analysis was by gas liquid chromatography (Perkin-Elmer Sigma 3D Chromatograph, Norwalk, CT).

For ruminal ammonia concentrations, 10 ml of the fresh ruminal liquor were transferred into plastic tubes. Samples were then acidified to pH less than 3 using 50% sulfuric acid and frozen until analyzed. Ammonia concentrations were determined by the phenol-hypochlorite reaction (Chaney and Marbach, 1962). Thawed samples were centrifuged at 45,000 x g for 20 min. The supernatant (10 μl) was reacted with 5 ml of phenol-hypochlorite reagent for direct reading by spectrophotometry (Spectronic 21, Bausch & Lomb, Rochester, NY). Preparations were allowed to react for 30 min in a water bath (39 C). Absorbance at 625 mμ was recorded. Appropriate standard curve was prepared using ammonium sulfate.

The remaining portion of the fresh ruminal liquor was frozen immediately for subsequent determination of insoluble fatty acid soap (IFAS) content by procedures modified from Jenkins and Palmquist (1982). Ruminal liquor samples (25 ml) in 50 ml screw-capped plastic tubes were centrifuged at 35,000 x g for 20 min. For soaked WCS samples, initial preparation required shredding to small pieces in a blender while still frozen. The supernatant was discarded, and the precipitate fraction was dried for 2 h at 80 C. Thirty milliliters of ethyl ether:acetone (1:1 v/v) was added and the contents were homogenized for 2 min. The tubes were capped, centrifuged at 600 x g for 10 min and the ether-acetone extract decanted into a 500 ml separatory funnel. Extraction with ether-acetone mixture was repeated twice more to remove all esterified (EFA) and nonesterified fatty acids (NEFA) leaving only the insoluble fatty acid soaps (IFAS). The ether-acetone extract in the separatory funnel was washed twice with 20 ml portions of .02 N potassium carbonate solution which resulted in separation of EFA (ether fraction) from NEFA (aqueous fraction). The ether fraction then was collected in a pretared 50 ml beaker. After the ether evaporated, sample was dried in the oven at 80 C for 2 h. The NEFA and IFAS fractions were acidified separately to pH less than 3 (by addition of 5 drops of concentrated sulfuric acid). Fractions were extracted separately using hexane (two 10-ml additions). Hexane extract was recovered in a pretared beaker and hexane was evaporated completely before samples were dried in the oven at 80 C for 2 h. Weight of the fatty acids recovered from the IFAS fractions divided by the total weight of fatty acids recovered from all three fractions equaled the proportion of the total fatty acids that soaped.

Data were analyzed by method of least-squares analysis of variance using general linear model procedures (SAS, 1982) with cow, period and treatment in the mathematical model (tables 15, 17, 19, 21, 23 and 24).

Results and Discussion

Trial I

Least-squares means for effects of dietary treatment factors on average 28-d body weight change and daily dry matter intake are in table 14; means for apparent dry matter, ADF and CP digestibilities are in table 16. Corresponding analyses of variance are in tables 15 and 17.

Table 14. Average Dry Matter Intake and Body Weight Change of Cows Fed Complete Diets Containing Intact Whole Cottonseed With or Without Calcium Hydroxide Treatment and Supplemented With Soybean Meal or Distiller's Dried Grains Plus Solubles (Trial I)

Item	Protein source				SE[c]	$Ca(OH)_2$[d] effect	Protein effect	$Ca(OH)_2$ x Protein interaction
	SBM[a]		DDGS[b]					
	+$Ca(OH)_2$	None	+$Ca(OH)_2$	None				
Dry matter intake, kg/d	8.5[e]	9.5	11.2	13.1	1.6	NS[f]	P<.1428	NS
Average body weight change, kg/28 d	4.7	-2.5	32.1	16.3	6.1	NS	P<.0767	NS

[a] SBM = Soybean meal.

[b] DDGS = Distiller's dried grains plus solubles.

[c] Standard error of mean.

[d] Calcium hydroxide.

[e] Values are least-squares means.

[f] NS = Not different (P>.15).

Table 15. Least-Squares Analysis of Variance for Effects of Calcium Hydroxide Treatment of Intact Whole Cottonseed and Protein Source on Dry Matter Intake and Body Weight Change (Trial I)

Source	df[a]		Dry matter intake, kg		Body weight change (kg/28 d)	
			MS[b]	P[c]	MS	P
Cow	3		3.430	.7096	4.148	.8764
Period	2		7.605	.4375	35.616	.2923
Treatment	3		10.963	.3563	56.364	.1943
$Ca(OH)_2$[d]		1	5.532	.4364	4.368	.6618
Protein		1	26.897	.1428	131.572	.0767
$Ca(OH)_2$xProtein		1	.460	.8129	33.152	.2750
Residual	3		6.896		18.676	

[a] Degrees of freedom.

[b] Mean square.

[c] Probability level.

[d] Calcium hydroxide.

Table 16. Coefficients of Apparent Digestibility of Dry Matter, Acid Detergent Fiber and Crude Protein of Complete Diets Containing Intact Whole Cottonseed With or Without Calcium Hydroxide Treatment and Supplemented With Soybean Meal or Distiller's Dried Grains Plus Solubles (Trial I)

Item	Protein source				SE[c]	$Ca(OH)_2$[d] effect	Protein effect	$Ca(OH)_2$ x Protein interaction
	SBM[a]		DDGS[b]					
	+$Ca(OH)_2$	None	+$Ca(OH)_2$	None				
	--------- Apparent digestibility, % ---------							
Dry matter	44.2[e]	48.7	37.9	45.5	1.4	$P<.0240$	$P<.0440$	NS[f]
Acid detergent fiber	28.2	37.7	32.7	41.0	1.3	$P<.0064$	$P<.0590$	NS
Crude protein	36.5	38.9	34.6	40.9	3.0	NS	NS	NS

[a] SBM = Soybean meal.

[b] DDGS = Distiller's dried grains plus solubles.

[c] Standard error of mean.

[d] Calcium hydroxide.

[e] Least-squares means.

[f] NS = Not different ($P>.15$).

Table 17. Least-Squares Analysis of Variance for Effects of Calcium Hydroxide Treatment of Intact Whole Cottonseed and Protein Source on Apparent Digestibility Coefficients for Diet Dry Matter (DMD), Acid Detergent Fiber (ADFD) and Crude Protein (CPD) (Trial I)

Source	df[a]	DMD		ADFD		CPD	
		MS[b]	P[c]	MS	P	MS	P
Cow	3	9.374	.3291	15.891	.1646	16.856	.6142
Period	2	94.230	.0221	108.147	.0143	216.382	.0547
Treatment	3	54.779	.0440	83.738	.0194	20.799	.5490
$Ca(OH)_2$[d]	1	97.106	.0238	210.217	.0064	51.996	.2395
Protein	1	60.863	.0435	39.921	.0590	.012	.9836
$Ca(OH)_2$xProtein	1	6.369	.3557	1.076	.6590	10.388	.5597
Residual	3	5.367		4.518		24.274	

[a] Degrees of freedom.

[b] Mean square.

[c] Probability level.

[d] Calcium hydroxide.

Effects of calcium hydroxide-treatment of WCS. There were no effects (P>.1500) of calcium hydroxide treatment of intact WCS on dry matter intake or average body weight change (tables 14 and 15). Pooled across protein sources there was a nonsignificant 12.8% reduction in dry matter intake due to calcium hydroxide treatment of WCS. A negative effect of alkali treatment of roughages on diet consumption (Capper et al., 1977; Saadullah et al., 1981) and on ruminal fermentation processes was reported (Capper et al., 1977). However, poor palatability (measured as lower intake) no longer was a problem when calcium hydroxide-soaked straws were washed in water before feeding (Saadullah et al., 1981). In their study calcium hydroxide-soaked feed materials constituted 60% of total diet whereas, in the present study, treated WCS constituted only 20% of total diet dry matter. Therefore, a palatability problem probably did not explain the trend of reduced intake where the WCS was treated with calcium hydroxide.

Pooled across dietary protein sources, calcium hydroxide treatment of intact WCS depressed apparent digestibility of dry matter (P<.0238) and acid detergent fiber (P<.0064, tables 16 and 17). Mean apparent dry matter digestibility was 6 percentage units lower in diets with calcium hydroxide-treated WCS than those with calcium hydroxide treatment (41.1 versus 47.1%, table 16). Acid detergent fiber apparent digestibility also was lower (8.8 percentage units) with calcium hydroxide treatment. Crude protein apparent digestibilities were not affected (P>.1500) by calcium hydroxide treatment.

Pooled across protein sources, mean apparent digestibilities of dry matter and acid detergent fiber of diets containing untreated WCS were 47.1 and 39.4%, respectively. These values were similar to those of Vernlund (1979) and Van Horn et al. (1984) whose diets contained CSH as roughage and supplemental fat at 2.5% of diet dry matter. Vernlund (1979) used a 1:1 mixture of hydrolyzed vegetable-animal fat and acidulated cottonseed soapstock, and reported apparent digestibilities of dry matter and acid detergent fiber at 43.0 and 27.4%, respectively. Van Horn et al. (1984) reported apparent digestibilities of 59.3% for dry matter and 38.3% for acid detergent fiber in a CSH-based diet with 2.5% added fat (a commercial blended, hydrolyzed fat).

The depression in digestibility due to calcium hydroxide treatment in the present study was not expected. Drackley et al. (1985), feeding 10% whole sunflower seed, compared the effectiveness of calcium hydroxide vs limestone as sources of dietary calcium in reversing depressions in fiber digestion. They reported that addition of calcium as limestone or calcium hydroxide resulted in digestibilities of NDF, ADF, hemicellulose and cellulose similar to a diet with SBM but with no added fat, indicating effectiveness of calcium addition. However, they did not find evidence of increased IFAS formation in the rumen with calcium addition from limestone or calcium hydroxide.

Treatment with calcium hydroxide has improved digestibility of low quality roughage (Klopfenstein et al., 1981; Saadullah et al., 1981); however, reports were not found where treatment actually depressed digestibility of fiber. Depressed digestibilities of dry matter and ADF in diets with calcium hydroxide-treated WCS in the present study were surprising, although treatment was for a different reason. There was suggestion of possible adverse effect of alkali treatment on dietary protein and amino acid nitrogen by converting them to forms which could not be utilized by ruminants (Capper et al., 1977). This might be a possible explanation for the depressed digestibilities reported here. Trends toward lower crude protein digestibility of diets with calcium hydroxide treatment of WCS (table 16) support this. About 24.5% of the total dietary protein in our experiment was contributed by WCS protein.

Effects of supplemental protein sources. Source of protein tended to produce differences in 28-d body weight changes ($P<.0767$, tables 14 and 15). Cows fed DDGS diets gained about 23 kg more than those fed the SBM-supplemental diet. Also cows fed diets with DDGS consumed 11% more dry matter than those receiving the SBM diets ($P<.1428$). Expressed as percent body weight, dry matter intake of cows averaged 1.7%. It is possible that cows fed DDGS-containing diets had greater gut-fill possibly contributing positively to body weight change. Digestibility data (to be discussed later) support the idea that gut-fill could have been greater with DDGS-supplemented diets than with SBM-based diets.

Soybean meal and DDGS as sources of supplemental protein resulted in differences in dry matter digestibility ($P<.0440$) and ADF digestibility ($P<.0590$), but not crude protein digestibility (tables 16 and 17). Digestibility of dietary dry matter (41.7 vs 46.5%) was depressed 10% with DDGS-compared with SBM-supplemented diets but ADF digestibility was improved slightly (33.0 vs 36.8%, P<.0590). There was no interaction of calcium hydroxide treatment of WCS with protein source (P>.1500) on any digestibility coefficients.

Use of DDGS as supplemental protein for low quality roughage diets (70% mature fescue hay) produced daily gains in lambs which were equal to those fed SBM-supplemented diets (Ely et al., 1982). Their diets were isonitrogenous with supplemental crude protein from DDGS and urea included at 13.8 and .1% of diet dry matter. In the present study, level of DDGS was 28.7% of dietary dry matter but no urea was included. Using body weight change as a response variable in the present experiment may not be the best measure of differences in diets because cows were nonlactating and mature.

Van Horn et al. (1985) reported lower nutritional value of DDGS as a source of supplemental protein compared with SBM in diets of lactating cows based on 50% corn silage (CS). Diets containing DDGS (ranging from 16 to 30% of the dry matter) caused reductions in milk yield and milk protein percent compared with SBM-controls but milk fat percent was higher. Associated with these effects were depressed digestibilities of organic matter and crude protein with DDGS. Their DDGS was thought to be heat-damaged severely (Van Horn et al., 1985). Effects of DDGS-supplementation on coefficients of apparent digestibility in our experiment were not consistent. We used nonlactating Holstein cows which consumed an average of 10.5 kg dry matter daily compared with about 21.0 kg reported by Van Horn et al. (1985). This may explain, partially, differences in digestibility results obtained between the two experiments. Also, the two experiments differed in the kind of roughage used (CSH plus grass hay in ours against CS in Van Horn's). It is possible that effects of DDGS on digestibility components differ with different kinds of roughage.

Trial II

Least-squares means of daily dry matter intake and body weight change are in table 18. Corresponding analyses of variance are in table 19. Calcium hydroxide treatment of crushed WCS caused a 13.6% depression in dry matter intake (11.0 vs 9.5 kg/d, $P<.0503$) but there were no differences in body weight change. Protein sources affected dry matter intake ($P<.0680$) but not body weight change. Mean dry matter intakes of diets supplemented with DDGS and SBM were 10.8 and 9.7 kg/d. Supplemental dietary protein affected dry matter intake similarly in trials I and II. There was no interaction between calcium hydroxide and supplemented protein source on dry matter intake or body weight change.

Coefficients of apparent digestion of dry matter, acid detergent fiber and crude protein are in table 20. Corresponding least-squares analyses of variance are in table 21. Inclusion in the diet of calcium hydroxide-treated WCS reduced coefficients of apparent digestion of dry matter ($P<.0878$) and ADF ($P<.1233$). Mean apparent digestibilities of dry matter and ADF were reduced 20.1 and 27.5%, respectively, with calcium hydroxide treatment. There was also a trend towards a reduced crude protein digestibility with calcium hydroxide treatment of WCS compared with no treatment ($P>.2032$).

Table 18. Average Daily Dry Matter Intake and Body Weight Change of Cows Fed Complete Diets Containing Crushed Whole Cottonseed With or Without Calcium Hydroxide Treatment and Supplemented With Soybean Meal or Distiller's Dried Grains Plus Solubles (Trial II)

Item	Protein source				SE[c]	$Ca(OH)_2$[d] effect	Protein effect	$Ca(OH)_2$ x Protein interaction
	SBM[a]		DDGS[b]					
	+$Ca(OH)_2$	None	+$Ca(OH)_2$	None				
Dry matter intake, kg/d	8.9[e]	10.5	10.0	11.5	.5	$P<.0503$	$P<.0680$	NS[f]
Average body weight change, kg/28 d	4.6	21.3	22.8	-1.0	8.5	NS	NS	NS

[a] SBM = Soybean meal.

[b] DDGS = Distiller's dried grains plus solubles.

[c] Standard error of mean.

[d] Calcium hydroxide.

[e] Least-squares means.

[f] NS = Not different ($P>.15$).

Table 19. Least-Squares Analysis of Variance for Effects of Calcium Hydroxide Treatment of Crushed Whole Cottonseed and Protein Source on Dry Matter Intake and Body Weight Change (Trial II)

Source	df[a]		Dry matter intake, kg		Body weight change (kg/28 d)	
			MS[b]	P[c]	MS	P
Cow	3		1.008	.1700	2.408	.8844
Period	2		1.033	.1622	12.068	.4914
Treatment	3		2.771	.0680	17.220	.4276
$Ca(OH)_2$[d]		1	3.676	.0503	.672	.8320
Protein		1	2.651	.0678	50.876	.1718
$Ca(OH)_2$xProtein		1	.018	.7939	.840	.8143
Residual	2		.199		11.648	

[a] Degrees of freedom.

[b] Mean square.

[c] Probability level.

[d] Calcium hydroxide.

Table 20. Coefficients of Apparent Digestibility of Dry Matter, Acid Detergent Fiber and Crude Protein of Complete Diets Containing Crushed Whole Cottonseed With or Without Calcium Hydroxide Treatment and Supplemented With Soybean Meal or Distiller's Dried Grains Plus Solubles (Trial II)

Item	Protein source				SE^c	$Ca(OH)_2$[d] effect	Protein effect	$Ca(OH)_2$ x Protein interaction
	SBM[a]		DDGS[b]					
	$+Ca(OH)_2$	None	$+Ca(OH)_2$	None				
	--------- Apparent digestibility, % ---------							
Dry matter	37.5[e]	49.3	37.5	44.6	2.5	$P<.0878$	NS[f]	NS
Acid detergent fiber	22.7	36.4	32.0	39.2	3.4	$P<.1233$	NS	NS
Crude protein	25.2	40.1	33.4	38.6	4.5	NS	NS	NS

[a] SBM = Soybean meal.

[b] DDGS = Distiller's dried grains plus solubles.

[c] Standard error of mean.

[d] Calcium hydroxide.

[e] Least-squares means.

[f] NS = Not different ($P>.15$).

Table 21. Least-Squares Analysis of Variance for Effects of Calcium Hydroxide Treatment of Crushed Whole Cottonseed and Protein Source on Apparent Digestibility Coefficients for Diet Dry Matter (DMD), Acid Detergent Fiber (ADFD), and Crude Protein (CPD) (Trial II)

Source	df[a]	DMD		ADFD		CPD	
		MS[b]	P[c]	MS	P	MS	P
Cow	3	6.180	.7586	2.603	.9534	9.835	.8816
Period	2	19.485	.4290	11.900	.6882	53.429	.4649
Treatment	3	48.637	.2399	114.914	.1916	85.772	.3700
$Ca(OH)_2$[d]	1	145.057	.0878	174.539	.1233	161.420	.2032
Protein	1	12.688	.4502	84.340	.2150	25.161	.5382
$Ca(OH)_2$xProtein	1	12.390	.4547	23.821	.4414	53.876	.3940
Residual	2	14.641		26.261		46.420	

[a] Degrees of freedom.

[b] Mean square.

[c] Probability level.

[d] Calcium hydroxide.

Similar trends for effects of calcium hydroxide treatment of dietary WCS and protein source on apparent digestibilities were noted in trials I and II. No significant effects of supplemental protein source or calcium hydroxide by protein source interaction on any of the digestion coefficients were detected (P>.150).

Ruminal liquor samples were obtained at the end of each period for 12 h (hourly sampling) following the 0730 h feeding. Main effect least-squares means for ruminal ammonia concentrations, pH and total VFA concentrations and molar proportions of acetate, propionate and butyrate are in table 22. Values are means pooled across cow, period and hour. Significantly higher concentrations of ammonia and total VFA, and higher ratios of acetate to propionate were in ruminal liquor of cows fed the calcium hydroxide-treated compared with untreated WCS (tables 23 and 24). Molar percentages of acetate and propionate were not affected significantly by calcium hydroxide. There were significant interactions of calcium hydroxide treatment by supplemental protein source on all the ruminal variables except molar percentages of propionate and butyrate (table 22).

Across protein source, calcium hydroxide treatment of WCS caused an increase in ruminal ammonia concentration by 19.6%. Increased ammonia concentration was greater with the SBM- than DDGS-supplemented diets (18 vs 11%). Comparing the two sources of supplemental proteins, it is apparent that SBM contained more than twice the ruminal degradable protein compared with DDGS (8.75 vs 3.70 mg/dl). This is to be expected because higher ruminal degradable

Table 22. Effects of Calcium Hydroxide Treatment of Dietary Crushed Whole Cottonseed and Protein Source on Ruminal Ammonia, pH and Volatile Fatty Acids (VFA) Concentrations (Trial II)

Ruminal variables	Protein source				SE[a]	$Ca(OH)_2$[a] effect	Protein effect	$Ca(OH)_2$ x Protein interaction
	SBM[a]		DDGS[a]					
	+$Ca(OH)_2$	None	+$Ca(OH)_2$	None				
Ammonia, mg/dl	9.5[b]	8.0	3.9	3.5	.2	$P<.05$	$P<.01$	$P<.05$
pH	6.7	6.6	6.7	6.9	.02	$P<.05$	$P<.01$	$P<.05$
Total VFA, mM	81.6	88.2	79.1	65.7	1.52	$P<.05$	$P<.05$	$P<.05$
Acetate:Propionate	3.56	3.56	2.90	2.46	.04	$P<.05$	$P<.01$	$P<.01$
	--------- moles/100 moles ---------							
Acetate	56.9	63.7	53.0	42.8	1.05	NS[c]	$P<.01$	$P<.05$
Propionate	16.0	18.2	18.5	17.5	.46	NS	NS	NS
Butyrate	8.8	6.3	7.6	5.4	.23	NS	NS	NS

[a] SBM = Soybean meal; DDGS = Distiller's dried grains plus solubles; $Ca(OH)_2$ = Calcium hydroxide; SE = Standard error of mean.

[b] Values are least-squares means.

[c] NS = Not different ($P>.15$).

Table 23. Least-Squares Analysis of Variance of Effects of Calcium Hydroxide Treatment of Dietary Crushed Whole Cottonseed and Protein Source on Ruminal Ammonia Concentration and pH (Trial II)

Source	df		Mean squares	
			Ammonia, mg/dl	pH
Cow	3		10.333*	.017^{NS}
Period	2		1.623^{NS}	.033^{NS}
Treatment	3		201.175**	.412**
Ca(OH)$_2$^{d}		1	16.779*	.164*
Protein		1	708.974**	.574**
Ca(OH)$_2$xProtein		1	7.286*	.272*
I[a]	3		.603	.008
Hour	11		8.748*	.045**
Cow x Hour	33		1.043^{NS}	.007^{NS}
Period x Hour	22		1.905^{NS}	.030**
Treatment x Hour	33		2.707^{NS}	.010^{NS}
Residual	21		.735	.007

[a] Pooled interaction (error term for cow, period and treatment).

^{NS} (P>.1); * (P<.05); ** (P<.01)

Table 24. Least Squares Analysis of Variance of Effects of Calcium Hydroxide Treatment of Dietary Crushed Whole Cottonseed and Protein Source on Concentrations of Ruminal Volatile Fatty Acids (Trial II)

Source	df	Mean square				
		Acetate	Propionate	Butyrate	Total VFA,[a] mM	Acetate:Propionate
		--------- moles/100 moles ---------				
Cow	3	128.120^{NS}	$.453^{NS}$	7.060^{NS}	156.138^{NS}	$.438^{*}$
Period	2	20.732^{NS}	5.676^{NS}	7.789^{NS}	92.959^{NS}	$.038^{NS}$
Treatment	3	1968.680^{*}	28.348^{NS}	42.524^{NS}	2515.309^{*}	7.739^{**}
Ca(OH)$_2$	1	53.443^{NS}	6.988^{NS}	105.236^{NS}	2822.770^{*}	$.898^{*}$
Protein	1	3956.026^{**}	22.148^{NS}	33.185^{NS}	3089.784^{*}	20.022^{**}
Ca(OH)$_2$xProtein	1	1851.504^{*}	56.236^{NS}	$.580^{NS}$	2476.737^{*}	1.407^{**}
I[b]	3	115.425	9.634	13.213	111.881	.036
Hour	11	118.414^{**}	23.371^{**}	6.124^{**}	311.851^{**}	$.144^{**}$
Cow x Hour	33	23.152^{NS}	4.357^{NS}	$.780^{NS}$	50.165^{NS}	$.048^{*}$
Period x Hour	22	25.550^{NS}	7.912^{**}	$.994^{+}$	66.974^{NS}	$.084^{**}$
Treatment x Hour	33	25.917^{NS}	3.141^{NS}	$.605^{NS}$	48.749^{NS}	$.037^{+}$
Residual	20	26.636	2.646	.520	50.328	.019

[a] VFA = Volatile fatty acids.

[b] I = Pooled interaction (error term for cow, period and treatment)

^{NS} $(P>.1)$; $^{+}$ $(P<.1)$; * $(P<.05)$; ** $(P<.01)$

protein in SBM than DDGS have been reported (Macleod and Grieve, 1983). The NRC (1985) estimated 38% ruminal protein degradability of DDGS compared to over 70% in SBM (Chalupa, 1975).

Total VFA also was improved with calcium hydroxide treatment of WCS, resulting in improved acetate:propionate ratio. Acetate production (molar percent) was improved by about 23.8% with calcium hydroxide treatment but was depressed with SBM-supplemented diets (10.7 percentage units lower). The big increase in acetate concentration probably reflected improved fiber digestion with the higher calcium on DDGS diets. It is not clear how this effect of calcium was produced. Diets with DDGS as protein supplements had a 20% lower ruminal acetate production than those with SBM-supplementation (47.9 vs 60.3 moles/150 moles). A lower acetate production as well as a lower acetate:propionate ratio suggested that the activity of the cellulolytic microbes were low which possibly was limited by the available ruminal ammonia. Ammonia concentration in ruminal liquor of 3.7 mg/dl is quite low for optimal activity of the cellulolytics.

Data from hourly samples of ruminal measurements were compared for differences in treatment responses over time of sampling. Significant differences among treatments were found only for ruminal concentrations of ammonia and molar proportions of acetate (table 25). These are illustrated in figures 12 and 13. Ruminal ammonia peaked around 2 to 4 h after feeding which was more pronounced with the SBM-supplemented diets.

Table 25. Tests of Heterogeneity of Regression of Ruminal Variables

Ruminal variables	Order of regression	Pooled		Partial		Difference		F
		df	MS	df	MS	df	MS	
Acetate, mol/100 mol	3	116	26.43	107	22.85	9	69.00	3.02*
Propionate, mol/100 mol	3	116	4.71	107	4.71	9	4.64	$.98^{NS}$
Butyrate, mol/100 mol	3	116	.79	107	.78	9	.84	1.07^{NS}
Total volatile fatty acids, mM	3	116	56.17	107	52.23	9	103.01	1.97^{NS}
Acetate/Propionate	4	116	.05	107	5.41	9	.10	1.99^{NS}
pH	4	116	.015	104	.014	12	.016	1.09^{NS}
NH_3, mg/dl	4	116	1.720	104	1.115	12	6.96	6.24**

Orthogonal Contrasts: Acetate: 1) Treatments 2 vs 4, 6 and 8^{NS}; 2) Treatments 4 vs 6 and 8**; 3) Treatments 6 vs 8**.

NH_3: 1) Treatments 2 vs 4, 6 and 8**; 2) Treatments 4 vs 6 and 8**; 3) Treatments 6 vs 8^{NS}.

Figure 12. Concentration of ammonia in ruminal liquor through 12 h postfeeding (Trial II).

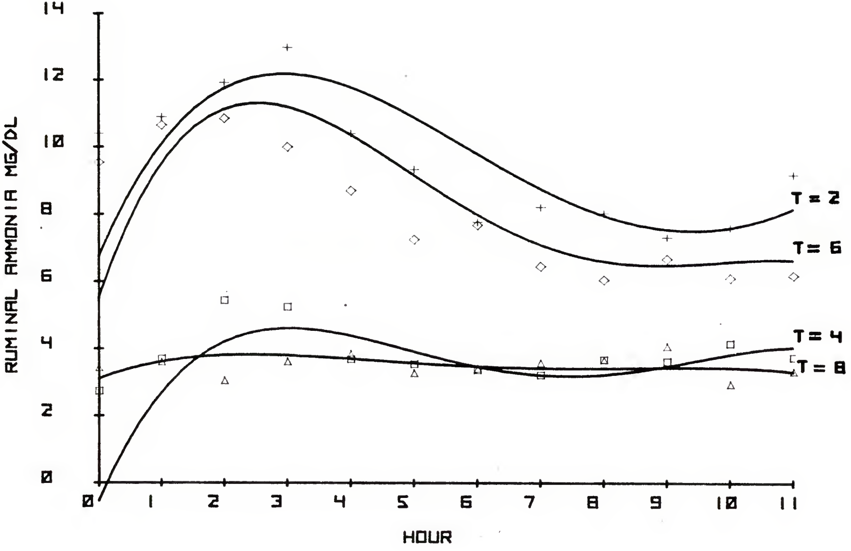
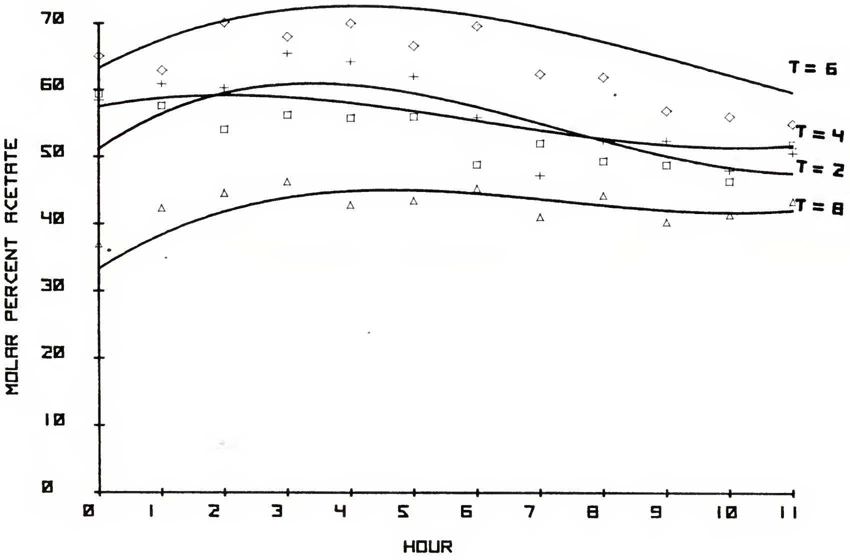

RUMINAL AMMONIA MG/DL
0
2
4
6
8
10
12
14
HOUR
0
1
2
3
4
5
6
7
8
9
10
11
T = 2
T = 6
T = 4
T = 8

Figure 13. Molar percent of acetic acid in ruminal liquor through 12 h postfeeding (Trial II).


70
60
50
40
30
20
10
0
MOLAR PERCENT ACETATE
0
1
2
3
4
5
6
7
8
9
10
11
HOUR
T = 6
T = 4
T = 2
T = 8

Laboratory Assessment of IFAS Formation. The main objective of calcium hydroxide treatment of WCS in the present experiment was to improve chance of insoluble fatty acid soap (IFAS) formation. Jenkins and Palmquist (1980) reported increased fiber digestibility along with a 30% increase in IFAS in the rumen of cows fed tallow and 2% limestone. Drackley et al. (1985) also reported alleviation of depressed fiber digestibility when calcium (either as limestone or calcium hydroxide) was added to a high-fat (from whole sunflower seed) diet. However, they did not report associated increases of IFAS formation in the rumen. Results of our laboratory experiment indicated that 51.89% more of total fatty acid extracted soaped when crushed WCS was soaked for 24 h in calcium hyroxide, compared with soaking in tap water only. Total fatty acids extracted were 4.1401 and 1.9915 g for treated and untreated WCS. Corresponding amounts of fatty acids separated as IFAS fraction were 1.2639 and .4003 g. In the subsequent in vivo experiment, we compared diets equal in calcium, supplement as calcium hydroxide in 22 h soaking or added as limestone supplement to the diet. There were no differences in intraruminal IFAS concentrations due to calcium treatment (60.3 vs 58.5% IFAS of total fatty acids extracted, for calcium hydroxide-treated WCS diet and for diets containing the conventional calcium source, table 26). Ruminal samples analyzed for IFAS were collected 4 h postfeeding. Our results indicated that, at .7% calcium in the diet (either as calcium hydroxide or limestone), there were substantial amounts of soaping in the rumen 4 h after feeding.

Table 26. Yields of Fatty Acids Extracted from Ruminal Liquor Samples

Diet number (description)	Total, g	Esterified and Nonesterified, g	Insoluble fatty acid soap (IFAS)	
			g	%[a]
2 (Calcium hydroxide-treated WCS[b] with SBM[b])	.1106	.0613	.0493	44.6
4 (Calcium hydroxide-treated WCS with DDGS[b])	.1342	.0323	.1019	75.9
6 (Untreated WCS with SBM)	.1202	.0685	.0517	43.0
8 (Untreated WCS with DDGS)	.1402	.0342	.1060	75.6

Note: Samples collected 4 h postfeeding.

[a] As percent of total fatty acids extracted from 20 ml ruminal liquor sample.

[b] WCS = Whole cottonseed; SBM = Soybean meal; DDGS = Distiller's dried grains with solubles.

However, calcium from the calcium hydroxide treatment of WCS did not cause any more soaping then those added as limestone feed supplement. A higher percent IFAS was formed when DDGS rather than SBM was included in the diet (75.7 vs 43.8%) perhaps attributed to by difference in proportions of calcium in the two protein supplements (.32 vs .08%, respectively).

General Discussion

Trials I and II differ only in the forms of WCS (intact and crushed) which were added to the diets either as calcium hydroxide-treated or untreated forms. Overall effects of calcium hydroxide treatment of WCS on intake and digestion coefficients were consistent between the two trials suggesting perhaps that crushing did not exert influence on the effects of calcium hydroxide treatment on variables studied.

We expected calcium hydroxide treatment of WCS to alleviate depressions in fiber digestibility by added fat in WCS better than limestone would, because of the relatively more soluble nature of the calcium hydroxide compared with limestone. However, no difference was evident from the results. It is possible that the level of WCS used (20% of diet dry matter) did not add high enough fat to cause problems with ruminal fiber digestion. Also, our diets were fairly high in fiber (40 to 44% ADF), which could have minimized potential depressive effects of fat on fiber digestibility (Palmquist and Conrad, 1980). If fiber digestion was not depressed to begin with, then there could not be alleviation with calcium hydroxide treatment.

CHAPTER V
RATE AND EXTENT OF DISAPPEARANCE OF COTTONSEED HULLS, GRASS HAY AND WHOLE COTTONSEED INCUBATED INTRARUMINALLY IN DACRON BAGS

Introduction

The rumen is the major site of breakdown of feeds in the gastrointestinal tract of ruminants. The processes of microbial degradation and fermentation convert much of the energy in plant fibers into useful metabolites which otherwise would not be available to the animal. Various techniques have been developed which aid in our understanding of these processes in the rumen. The in situ technique has gained acceptance as a method of estimating digestibility in the rumen. The method offers a relatively simple procedure (Mehrez and Orskov, 1977) for quantifying ruminal degradation of feedstuffs, dietary components and specific nutrients. Its biggest advantage perhaps is applicability to measurement of rates of digestion in vivo. Numerous studies have used the in situ technique for the evaluation of various kinds of feedstuffs including concentrates (Figroid et al., 1972; Weakley et al., 1983; Ha and Kennelly, 1984), forages (Burton et al., 1967; Uden et al., 1974; Van Hellen and Ellis, 1977) and by-product roughages (Neathery, 1969; Belyea et al., 1979).

Changes in composition of the diet due to supplementation could affect digestibility. Abou Akkada and El-Shazly (1958) and El-Shazly et al. (1961) reported that increasing the amount of starch in the diet affected cellulose digestion in vitro and in vivo. Also, in situ dry matter digestion of forages was affected by the type of diet fed (Van Keuren and Heinemann, 1962; Neathery, 1969; Lindberg, 1981). Other ingredients introduced into the diet likely also would affect ruminal digestion.

Therefore, in conjunction with trial II (Chapter IV), we conducted an in situ experiment to study the effect of calcium hydroxide treatment of dietary crushed whole cottonseed and sources of supplemental protein on the rate and extent of in situ ruminal disappearance of dry matter and acid detergent fiber of cottonseed hulls and grass hay, and extent of dry matter disappearance of intact whole cottonseed in dacron bags.

Materials and Methods

Four nonlactating Holstein cows fitted with permanent ruminal cannulae were used. Cows were assigned randomly to dietary treatment sequences in a balanced incomplete block experiment consisting of three 28-d periods. Diets, feeding and management of the cows were described previously (Chapter IV, trial II). Dietary treatment factors were crushed WCS (20% of diet dry matter) which have been treated with calcium hydroxide or not and dietary protein sources (DDSG vs SBM). Full explanation of treatment formulations are in Chapter IV.

Dacron polyester material (N. Erlanger, Blumgardt & Co., Inc., NY) with pore sizes between 20 to 70 μm was used. Bags were made by cutting out 19x19 cm square pieces. Each piece was flamed along all edges, folded in half and sewn with a double row of stitches along two adjacent cut edges using 100% polyester thread. Needle holes were sealed with clear water-proof glue (VLP Liquid Vinyl, P.D.I. Inc., St. Paul, MN). Final internal dimensions of bags were 15.5x7.5 cm.

Five-gram samples of cottonseed hulls (CSH) and grass hay each ground through a 2 mm screen and intact whole cottonseed (WCS) were weighed into previously tared bags. Grass hay used was from a mixed pasture of approximately 65% bahiagrass, 30% common bermudagrass and 5% other species. Dry matter of each feedstuff was determined each time samples were weighed into bags. Bags were closed individually and tied with a 30 cm long nylon twine. The free end of the twine securing each bag was anchored to a 100 cm length steel chain, one in the rumen of each cow. One end of the steel chain was secured to the cannula for each access when adding bags at varying incubation intervals. The steel chain served to hold down the sample bags below the surface of the ruminal contents.

Incubation intervals for CSH and grass hay samples were 0, 2, 4, 8, 12, 24, 36, 48 and 72 h. For WCS, only the 48 and 72 h intervals were of interest for estimating extent of dry matter disappearance. Bags were added to the ventral rumen at respective time intervals and all removed at once at the end of the incubation. The 0 h bags were put in the rumen for 5 min, removed and handled as bags incubated for longer intervals. Upon removal from the rumen, all bags were rinsed by dipping in a bucket of tap water and immediately placing on ice.

The nylon twine securing bags to the chain was cut about 5 cm from the bag to avoid entanglement during the washing cycle. Washing was in a commercial washing machine using the cold wash regular rinse cycle (30 min). Bags were then dried at 60 C for 48 h. Bags were weighed to determine the dry matter remaining. Residues in the bags were analyzed for acid detergent fiber content (Goering and Van Soest, 1970).

The percent disappearance of dry matter and acid detergent fiber (ADF) at each incubation interval was calculated by difference using the proportion remaining at the end of the incubation interval. Dry matter and ADF disappearance kinetics were estimated using a model previously employed by Orskov and McDonald (1979):

$$D = A + B\,(1 - e^{-kt})$$

where D = Disappearance of nutrient at time t, %

A = Rapidly soluble fraction, %

B = Potentially digestible insoluble fraction, %

k = Rate constant of digestion of fraction B, /h

t = Time of incubation in the rumen, h.

Nonlinear estimates of A, B, and k and lag time (i.e., time interval between introduction of bags into the rumen and commencement of disappearance of fraction B) were estimated by an iterative least-squares procedure based on Marquardt's compromise as performed by nonlinear procedures of the Statistical Analysis System (SAS, 1982). Estimates of rate constant (k), lag time and extent of disappearance of fraction B at the end of the 72-h incubation were analyzed by method of least-squares analysis of variance using general linear model procedures of SAS (1982). Cow, period and treatment were in the mathematical model (table 31). One cow was removed from the experiment after the end of the second period because of health reasons unrelated to the dietary treatments.

Results and Discussion

Chemical composition of the roughages used are in table 13 (Chapter IV). Estimated TDN for CSH and grass hay were 51.6 and 54.0%. Values were estimated from regression equations originally derived from the analyzed chemical composition of similar feedstuff types (New York Dairy Herd Improvement Forage Testing Laboratory, Ithaca, NY).

Results of dry matter disappearances of CSH, grass hay and WCS from dacron bags incubated in the rumen are in table 27. Each value is the overall pooled mean for that incubation interval (irrespective of dietary treatment factors) based on 11 cow-period observations. Final extent (after 72 h) of dry matter disappearances of CSH, grass hay and WCS were 58.3, 42.3 and 28.8%, respectively. Lower 72-h dry matter disappearance of the grass hay than CSH indicated a fairly poor quality of the hay. Cottonseed hulls frequently contain between 10 and 15% lint on dry weight basis (Vernlund, 1979). Lint is almost entirely native cellulose, which is completely digestible. Cellulose also occurs in the bran fraction of the CSH, and together they

Table 27. Dry Matter Disappearance from Cottonseed Hulls, Grass Hay and Intact Whole Cottonseed Incubated in Dacron Bags in the Rumen

Incubation time, h	Cottonseed hulls	Grass hay	Whole cottonseed[a]
	%		
2	8.6 ± 1.0[b]	9.1 ± 0.4	
4	9.5 ± 0.8	9.9 ± 1.4	
8	11.7 ± 1.4	11.8 ± 0.6	
12	15.5 ± 1.0	14.9 ± 0.4	
24	29.5 ± 1.9	23.5 ± 1.0	
36	40.8 ± 0.7	30.0 ± 0.8	
48	49.0 ± 1.5	35.9 ± 0.4	21.4 ± 1.9
72	58.3 ± 1.6	42.3 ± 0.7	28.8 ± 1.5

[a] Samples were not treated and not crushed.

[b] Means of 11 cow-period observations ± standard deviation.

constitute about 44% of the dry weight of CSH (Dunning, 1948). Other major constituents are pentosans (29.5%) and lignins (21.9%).

Neathery (1969) reported 36.5% in situ dry matter disappearance of CSH after 72-h incubation in the rumen of a fistulated Guernsey steer fed Coastal bermudagrass hay. When the diet was changed to 50:50 alfalfa:orchardgrass hay, the percentage dry matter disappearance of CSH was 32.3% at the end of 72-h incubation. Both of these values were considerably lower than values in the present study. One possible reason attributable to the difference is the composition of the basal diets. Our diets contained 50% concentrate and 50% roughage. Cottonseed hulls contributed 50% of the roughage in the diet which perhaps stimulated a microbial fermentation that is more favorable to CSH digestion than in Neathery's (1969) experiment. Our basal diets also contained more other fermentable constituents (i.e., protein and soluble carbohydrates) which could enhance microbial fermentation. Stimulation of cellulose digestion was reported when easily fermentable carbohydrate was 5 to 10% of the diet (Burroughs et al., 1950). Increased in situ dry matter disappearance also has been demonstrated with increasing proportion of concentrate in the diet (Weakley et al., 1983).

Dry matter disappearance (72-h incubation) of grass hay (42.3%) in our study (table 27) compared closely with that of coastal bermudagrass hay (44.5%) of Neathery (1969). Comparisons such as this must be interpreted with caution because of the possibility of large differences in experimental conditions. Nevertheless, it could provide an empirical comparison of the relative value of the forages. Crude protein content of the bermudagrass hay (Neathery, 1969) was 7.4% compared with 8.5% for the grass hay in our experiment. Morantes reported a 60% dry matter disappearance of Floralta limpograss (2.6% protein) after 72-h incubation.

Whole cottonseed dry matter disappearance after the 72-h incubation was 28.8%. Visual assessment revealed a denuded seed coat from which all the lint had been digested away during the 72-h incubation. Thus, the percent lint of WCS in our study could be estimated at about 29% of the dry weight. Judged by the appearance of the WCS residue remaining after 48 h not all the lint had been digested. Approximately 27% of the potentially digestible lint, after 72 h of incubation, remained at the end of 48-h incubation (dry matter disappearances of 21 vs 29% for 48-h and 72-h incubations).

There was no evidence of disintegration of the seed coat due to microbial degradation in the bags after the 72-h incubation. Palmquist (1984) similarly reported no sign of microbial degradation of the seed coat of WCS after 48-h ruminal incubation in dacron bags. This suggests that breaking the seed coat is essential before nutrients contained within the seed coat would be available to the ruminal microbes. Cows probably achieved this by chewing the seeds. Coppock et al. (1985a) found that, devoid of lint, a higher percentage (11 vs .7%) of WCS escaped chewing and subsequent digestion along the entire digestive tract of dairy cows than if it still had lint. Seeds were recovered in the feces that retained the nutritional composition of WCS before consumption (Coppock et al., 1985a).

Acid detergent fiber disappearance values are in table 28. Extents of ADF disappearance after 72-h incubation in the rumen were 48.4 and 45.8% for CSH and grass hay. Table 29 shows the mean rate constants and lag times of disappearance of dry matter and ADF from CSH and grass hay incubated in the rumen. Values are least-squares means based on 11 cow-period observations, pooled across dietary treatments. Least-squares analyses of variance are in table 31. Dry matter disappearance rate constants for CSH and grass hay were .0394 and .0353/h, respectively. There are few rate constants for digestion of low quality roughages in the literature. Floralta limpograss hay had a rate constant for dry matter disappearance of .0480/h (Morantes, 1986).

Effects of dietary treatments on extent of disappearance rate constants and lag times of grass hay from the rumen are in tables 30 and 32 for dry matter and ADF, respectively. Analyses of variance are in tables 31 and 33.

Calcium hydroxide treatment improved (P<.0620) 72-h extent of ruminal dry matter disappearance of grass hay (average 45.0 vs 39.2%, table 30). There also was an 8% improvement in extent of ADF disappearance, though not different (P>.1500) from diets without calcium hydroxide treated-WCS (table 32). Calcium hydroxide treatment by protein source interactions were not detected for 72-h extent of disappearance of dry matter or ADF.

Extent of disappearance of grass hay dry matter was 8.3% higher (P<.1010) with SBM-compared with DDGS-supplemented diets (table 30). Lower ruminal ammonia concentrations reported earlier

Table 28. Acid Detergent Fiber Disappearance from Cottonseed Hulls and Grass Hay Incubated in Ruminal Dacron Bags

Incubation time, h	Cottonseed hulls	Grass hay
	%	%
2	3.0 ± 0.6[a]	10.2 ± 0.9
4	4.3 ± 1.1	11.1 ± 0.7
8	7.5 ± 2.0	12.9 ± 0.8
12	8.4 ± 0.7	18.3 ± 1.2
24	20.2 ± 1.9	25.4 ± 0.4
36	31.0 ± 1.3	34.0 ± 0.9
48	39.3 ± 2.1	40.0 ± 0.9
72	48.4 ± 2.0	45.8 ± 1.1

[a] Means of 11 cow-period observations ± standard deviation.

Table 29. Mean Rate Constants and Lag Times of Ruminal Disappearance of Dry Matter and Acid Detergent Fiber from Cottonseed Hulls and Grass Hay

Variables	Cottonseed hulls		Grass hay	
	Dry matter	ADF[a]	Dry matter	ADF
Rate constant, /h	.0394±.0079[b]	.0369±.0089	.0353±.0006	.0372±.0027
Lag time, h	7.01±1.61	8.26±2.31	5.06±.16	5.81±.47

[a] Acid detergent fiber.

[b] Values are overall least-squares means ± standard error.

Table 30. Effects of Calcium Hydroxide Treatment of Dietary Crushed Whole Cottonseed and Protein Source on Rate Constants, Lag Times and Extents of In Situ Ruminal Disappearance of Grass Hay Dry Matter

Item	Protein source				SE^a	$Ca(OH)_2{}^a$ effect	Protein effect	$Ca(OH)_2$ x Protein interaction
	SBM^a		$DDGS^a$					
	$+Ca(OH)_2$	None	$+Ca(OH)_2$	None				
Extent of disappearance after 72 h, %	46.47[b]	41.40	43.49	37.04	1.27	$P<.0621$	$P<.1006$	NS
Rate constant, /h	.0326	.0377	.0375	.0342	.0006	NS[c]	NS	$P<.0209$
Lag time, h	5.64	5.04	5.70	3.84	.16	$P<.0237$	$P<.0721$	$P<.0591$

[a] SBM = Soybean meal; DDGS = Distiller's dried grains plus solubles; $Ca(OH)_2$ = Calcium hydroxide; SE = Standard error of mean.

[b] Values are least-squares means.

[c] NS = Not different ($P>.15$).

Table 31. Least-Squares Analysis of Variance of Effect of Calcium Hydroxide Treatment of Dietary Crushed Whole Cottonseed and Protein Source on Lag Times, Rate Constants and 72-H Extents of Ruminal Disappearance of Grass Hay Dry Matter Incubated in Dacron Bags

Source	df[a]	Extent at 72 h, %		Rate constant, /h		Lag time, h	
		MS[a]	P<F[a]	MS	P<F	MS	P<F
Cow	3	4.113	.5010	$1.4x10^{-5}$	.0599	.710	.0784
Period	2	2.790	.5660	$1.2x10^{-5}$	.0694	.786	.0705
Treatment	3	38.146	.0883	$1.5x10^{-5}$	.0563	.875	.0310
$Ca(OH)_2$[d]	1	53.176	.0621	$1.2x10^{-6}$	.3585	2.425	.0237
Protein	1	30.818	.1006	$1.0x10^{-6}$	.3930	.738	.0721
$Ca(OH)_2$xProtein	1	1.090	.6390	$4.1x10^{-5}$	.0209	.921	.0591
Residual	2	3.638		$8.8x10^{-7}$		.060	

[a] df = Degrees of freedom; MS = Mean square; P<F = Significance level.

Table 32. Effects of Calcium Hydroxide Treatment of Dietary Crushed Whole Cottonseed and Protein Source on Rate Constants, Lag Times and Extents of In Situ Ruminal Disappearance of Grass Hay Acid Detergent Fiber

Item	Protein source							
	SBM[a]		DDGS[a]		SE[a]	$Ca(OH)_2$[a] effect	Protein effect	$Ca(OH)_2$ x Protein interaction
	+$Ca(OH)_2$	None	+$Ca(OH)_2$	None				
Extent of disappearance after 72 h, %	48.83[b]	45.77	46.44	41.98	1.46	NS[c]	NS	NS
Rate constant, /h	.0353	.0351	.0419	.0357	.0027	NS	NS	NS
Lag time, h	5.81	6.71	5.44	5.56	.48	NS	NS	NS

---

[a] SBM = Soybean meal; DDGS = Distiller's dried grains plus solubles; $Ca(OH)_2$ = Calcium hydroxide; SE = Standard error of mean.

[b] Values are least-squares means.

[c] NS = Not different ($P > .15$).

Table 33. Least-Squares Analysis of Variance of Effect of Calcium Hydroxide Treatment of Dietary Crushed Whole Cottonseed and Protein Source on Lag Times, Rate Constants and 72-H Extents of Ruminal Digestion of Acid Detergent Fiber of Grass Hay Incubated in Dacron Bags

Source	df[a]	Extent at 72 h, %		Rate constant, /h		Lag time, h	
		MS[a]	P<F[a]	MS	P<F	MS	P<F
Cow	3	5.812	.4835	$1.4x10^{-4}$	.1103	1.283	.2963
Period	2	.103	.9790	$9.3x10^{-5}$	.1527	.159	.7611
Treatment	3	20.414	.1971	$2.5x10^{-5}$	.4250	.533	.5218
$Ca(OH)_2$[d]	1	22.620	.1629	$1.7x10^{-5}$	.4246	.422	.4582
Protein	1	21.718	.1679	$2.9x10^{-5}$	.3181	1.330	.2470
$Ca(OH)_2$xProtein	1	1.120	.6775	$2.1x10^{-5}$	.3807	.339	.5000
Residual	2	4.825		$1.7x10^{-5}$		.508	

[a] df = Degrees of freedom; MS = Mean square; P<F = Significance level.

(table 22, Chapter IV) suggested that perhaps ruminal ammonia was limiting for growth of the microbial population with the DDGS diets.

Lag time of grass hay dry matter disappearance showed evidence of a temporary inhibition ($P<.0237$) due to calcium hydroxide treatment of dietary WCS (table 30). Such evidence was not apparent for ADF disappearance (table 31).

Interactions existed between calcium hydroxide treatment of WCS and protein source for the rate constants ($P<.0209$) and lag times ($P<.0591$) of grass hay dry matter disappearance. Figures 14 and 15 illustrate these interactions. With calcium hydroxide-treated WCS, grass hay dry matter disappearance rate was retarded with SBM as the supplemental protein source compared with no treatment of WCS, whereas in diets with DDGS as protein supplement, the rate was enhanced by treatment of WCS with calcium hydroxide, compared with no treatment. The calcium hydroxide-treated WCS also delayed the initiation of ruminal degradation (higher lag times) of grass hay dry matter ($P<.0237$). Lag time was 1.23 h longer with calcium hydroxide treatment of WCS than without. Protein source also affected lag time of dry matter disappearance from fraction B, with SBM-supplemented diets having a .57 h longer lag time than DDGS-supplemented diets. Figure 15 illustrates the interaction of WCS treatment by protein source ($P<.059$) on lag time of dry matter disappearance of hay from fraction B. With no calcium hydroxide treatment of WCS, lag time was 1.20 h longer for SBM- than DDGS-supplemented diets; however, protein source had little effect on lag times of dry matter disappearance of hay when dietary WCS was treated with calcium hydroxide (average lag

Figure 14. Effect of dietary treatment on rate constants of grass hay dry matter disappearance.

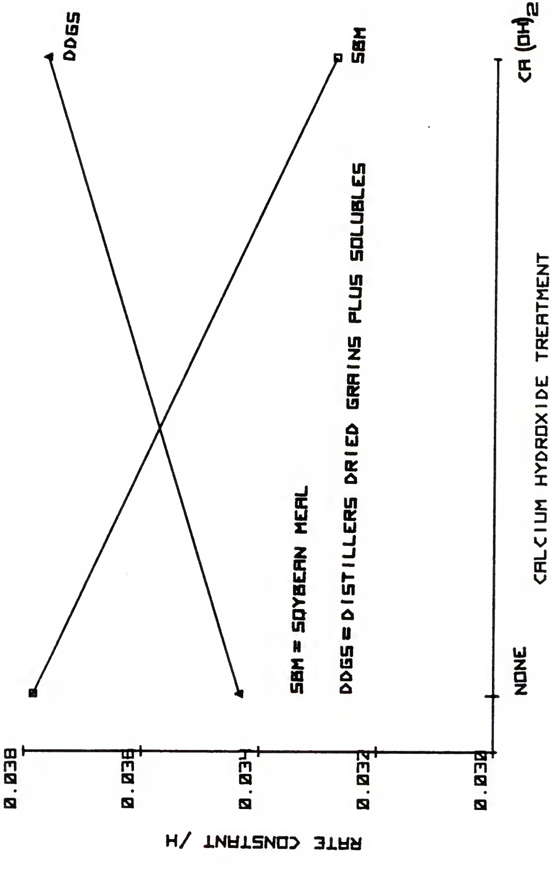
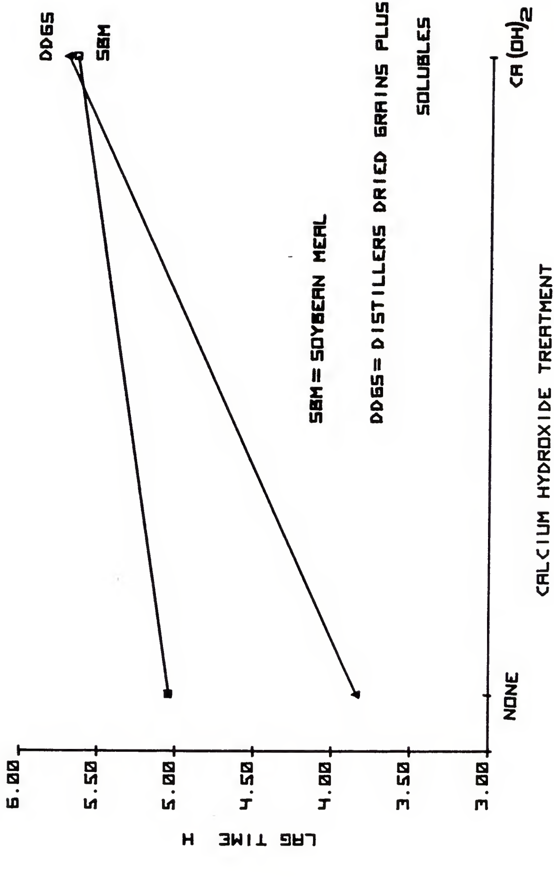

RATE CONSTANT /H
0.038
0.038
0.034
0.032
0.030
NONE
CA (OH)$_2$
CALCIUM HYDROXIDE TREATMENT
DDGS
SBM
SBM = SOYBEAN MEAL
DDGS = DISTILLERS DRIED GRAINS PLUS SOLUBLES

Figure 15. Effect of dietary treatment on lag times of grass hay dry matter disappearance.


LAG TIME H
6.00
5.50
5.00
4.50
4.00
3.50
3.00
NONE
CA (OH)$_2$
CALCIUM HYDROXIDE TREATMENT
DDGS
SBM
SBM= SOYBEAN MEAL
DDGS= DISTILLERS DRIED GRAINS PLUS
SOLUBLES

time = 5.67 h). Overall, lag time was greater with calcium hydroxide treatment of WCS than without (5.67 vs 4.44 h). Neither dietary fiber affected any of the disappearance measurements associated with degradation of ADF of the grass hay (tables 32 and 33).

Effect of dietary treatments on disappearance of cottonseed hull dry matter with ruminal incubation is in table 34 and analyses of variance of the data are in table 35. There were no detectable differences due to either calcium hydroxide or protein source (P>.1500) for rate constants, lag times or 72-h extents of disappearance. Similarly, there was no evidence of a calcium hydroxide by protein source interaction (P>.1500).

In table 36 are least-squares means for rate constants, lag times and extents of cottonseed hull ADF disappearance from the in situ ruminal incubation. Least-squares analyses of variance are in table 37. Calcium hydroxide treatment did not affect rate constants, lag times or extents of disappearance (P>.1500). Protein source did not affect rate constants or lag times of disappearance but caused a slight effect on extent of disappearance of cottonseed hull ADF (P<.1450). Extent of disappearance was slightly higher (3.95 percentage units) when DDGS rather than SBM was the supplemental protein in the diet.

Although treatment effects on estimates of disappearance kinetics were not significant (tables 34 and 35), data appear to suggest a trend towards a negative effect on disappearance of dry matter and ADF from CSH due to calcium hydroxide treatment of WCS.

Table 34. Effects of Calcium Hydroxide Treatment of Dietary Crushed Whole Cottonseed and Protein Source on Rate Constants, Lag Times and Extents of In Situ Ruminal Disappearance of Cottonseed Hull Dry Matter

Item	Protein source				SE[a]	$Ca(OH)_2$[a] effect	Protein effect	$Ca(OH)_2$ x Protein interaction
	SBM[a]		DDGS[a]					
	+$Ca(OH)_2$	None	+$Ca(OH)_2$	None				
Extent of disappearance after 72 h, %	52.38[b]	60.48	60.90	60.28	2.54	NS[c]	NS	NS
Rate constant, /h	.0271	.0447	.0410	.0466	.0079	NS	NS	NS
Lag time, h	8.13	6.13	6.98	6.50	1.61	NS	NS	NS

[a] SBM = Soybean meal; DDGS = Distiller's dried grains plus solubles; $Ca(OH)_2$ = Calcium hydroxide; SE = Standard error of mean.

[b] Values are least-squares means.

[c] NS = Not different (P>.15).

Table 35. Least-Squares Analysis of Variance of Effect of Calcium Hydroxide Treatment of Dietary Crushed Whole Cottonseed and Protein Source on Lag Times, Rate Constants and 72-H Extents of Ruminal Digestion of Dry Matter of Cottonseed Hull Incubated in Dacron Bags

Source	df[a]	Extent at 72 h, %		Rate constant, /h		Lag time, h	
		MS[a]	P<F[a]	MS	P<F	MS	P<F
Cow	3	24.539	.3918	$2.7x10^{-4}$	.6492	1.829	.8180
Period	2	20.423	.4147	$.9x10^{-5}$	.9683	10.209	.3622
Treatment	3	40.332	.2750	$5.2x10^{-4}$	.4718	1.901	.8107
$Ca(OH)_2$[d]	1	22.320	.3401	$2.2x10^{-4}$	.3378	.243	.8566
Protein	1	39.567	.2400	$1.4x10^{-4}$	.4162	3.506	.5181
$Ca(OH)_2$xProtein	1	43.463	.2252	$.8x10^{-4}$	.5218	1.317	.6806
Residual	2	14.467		$1.4x10^{-4}$		5.797	

[a] df = Degrees of freedom; MS = Mean square; P<F = Significance level.

Table 36. Effects of Calcium Hydroxide Treatment of Dietary Crushed Whole Cottonseed and Protein Source on Rate Constants, Lag Times and Extents of In Situ Ruminal Disappearance of Cottonseed Hull Acid Detergent Fiber

Item	Protein source							
	SBM[a]		DDGS[a]		SE[a]	$Ca(OH)_2$[a] effect	Protein effect	$Ca(OH)_2$ x Protein interaction
	+$Ca(OH)_2$	None	+$Ca(OH)_2$	None				
Extent of disappearance after 72 h, %	43.15[b]	49.73	50.67	50.10	2.56	NS	P=.1450	NS
Rate constant, /h	.0286	.0391	.0375	.0432	.0134	NS[c]	NS	NS
Lag time, h	10.06	6.93	9.44	6.18	3.46	NS	NS	NS

---

[a] SBM = Soybean meal; DDGS = Distiller's dried grains plus solubles; $Ca(OH)_2$ = Calcium hydroxide; SE = Standard error of mean.

[b] Values are least-squares means.

[c] NS = Not different (P>.15).

Table 37. Least-Squares Analysis of Variance of Effect of Calcium Hydroxide Treatment of Dietary Crushed Whole Cottonseed and Protein Source on Lag Times, Rate Constants and 72-H Extents of Ruminal Digestion of Acid Detergent Fiber of Cottonseed Hull Incubated in Dacron Bags

Source	df[a]	Extent at 72 h, %		Rate constant, /h		Lag time, h	
		MS[a]	P<F[a]	MS	P<F	MS	P<F
Cow	3	15.000	.3180	$1.1\text{x}10^{-4}$	.6853	9.079	.6118
Period	2	9.824	.3996	$1.1\text{x}10^{-5}$	.9445	19.095	.3853
Treatment	3	30.912	.1795	$.9\text{x}10^{-4}$	.7218	9.029	.6132
$Ca(OH)_2$[d]	1	14.472	.2752	$1.0\text{x}10^{-4}$	.5266	.006	.9838
Protein	1	35.629	.1447	$9.5\text{x}10^{-5}$	.5420	1.073	.7929
$Ca(OH)_2$xProtein	1	29.264	.1687	$1.2\text{x}10^{-5}$	.8171	23.213	.2984
Residual	2	6.540		$1.8\text{x}10^{-4}$		11.970	

[a] df = Degrees of freedom; MS = Mean square; P<F = Significance level.

This was suggested by the smaller rate constants and longer lag times with calcium hydroxide treatment.

CHAPTER VI
SUMMARY, DISCUSSION AND CONCLUSIONS

Feeding of WCS to dairy cows was studied in three experiments to evaluate effects of added fat from WCS on milk production and on diet digestibility. The first experiment was designed to study effects of levels of WCS (0, 15 and 30% of dry matter), levels of calcium (.69 and 1.29%) from added limestone and sources of roughage (CSH, AH and CS), and the potential interactions of these variables on production performance and diet digestibility. Diets were formulated with equal roughage content (40%) and 18% crude protein. Thirty-six mid-lactation Holstein cows were used in a balanced incomplete block design involving three 35-d periods. The internal marker, permanganate lignin, was used to compare digestibility of total dietary dry matter and fiber components.

With increasing dietary WCS, there was a linear decrease in dry matter intake ($P<.0001$). Both actual ($P<.0015$) and FCM yields ($P<.0001$) responded in a curvilinear fashion to increasing WCS with higher responses with the 15% WCS than 0 or 30% WCS. Yields of milk fat and milk protein declined with WCS inclusion at 30% in the diet. Whole cottonseed at 30% of the diet dry matter was higher than needed for cows in the present experiment for maximum lactational performance. The higher level of WCS addition did not affect digestibility of dry matter but increased (linear) digestibilities of dietary NDF

($P<.0252$) and ADF ($P<.0061$). Decreases in milk fat percent (linear, $P<.0001$) and yield (curvilinear, $P<.0014$) suggested possible depressing effects of added fat from WCS on fiber digestibility. If that were true, then our technique for estimating digestibility (using permanganate lignin as marker) did not reflect digestibility differences accurately. When analyzing for permanganate lignin, all samples were treated similarly regardless of possible differences in lignin contents among samples. Although actual lignin contents were not determined, it is believed that 90-minute oxidation time of lignins by potassium permanganate may not have been long enough for samples high in lignin contents specifically fecal and feed samples from the high-WCS, CSH-based diets. Gossypol toxicity was not an apparent problem with the WCS-containing diets. There was no effect of dietary calcium level on any of the response variables except milk protein which was slightly depressed at the higher calcium level ($P<.0334$). Roughage sources affected all production variables except yields of milk fat and FCM. Dry matter intake was highest with CSH-based diets ($P<.0001$). Corn silage-based diets were highest in energy concentration and, in spite of a 12.5% lower intake with CS-based diets than either CSH- or AH-based diets, intake of NE_1 was nearly equal from all diets. Cottonseed hull-based diets produced highest milk yield but lowest milk fat and protein percents. Highest milk fat percent was with CS- and AH-based diets. Highest milk protein was with diets containing CS. There was no effect of roughage source on fiber digestibility. Lowest dry matter digestibility was with AH-based diets. Significant calcium by roughage interaction existed affecting dry matter intake ($P<.0540$), milk fat percent ($P<.0504$), milk protein percent ($P<.0104$) and all coefficients of apparent digestibility measured.

It is evident from results of this experiment that 30% level of WCS inclusion in the diet proved too high and that it affected performance adversely. Under conditions similar to the present experiment, 15% WCS in the diet was the best. The higher level of calcium added as limestone was not beneficial except in diets based on CSH where it improved milk fat and milk protein percents and digestibilities of NDF and ADF in the diet.

The second experiment was to study effects of prefeeding treatment of WCS with calcium hydroxide to increase ruminal soaping of fatty acids, and to compare value of DDGS with SBM as sources of supplemental protein in diets containing 20% WCS (crushed or intact). Treatments were in a 2x2 factorial arrangement: with or without calcium hydroxide treatment factored with DDGS or SBM. Eight nonlactating cows (4 fitted with ruminal cannulae) were used in a balanced incomplete block experiment (three 28-d periods). Diets consisted of a 25% CSH, 25% grass hay, 20% WCS and 30% concentrate mix. Diets were formulated to contain 16% crude protein and 70% TDN and fed *ad libitum*. Digestibility estimation was by ratio technique using chromic oxide as an indigestible marker in two 5 g doses daily for 10 d. Fecal grab samples were taken the last 5 d. Ruminal liquor samples were collected hourly for 12 h to monitor changes in ruminal metabolites. Calcium hydroxide treatment of WCS depressed coefficients of apparent digestibility of dry matter and ADF, whereas treatment was intended to help alleviate depression of fiber digestion through a soaping reaction with fatty acids. Although substantial soaping was detected in ruminal liquor samples, there was no evidence of alleviation of decreased fiber digestibility due to calcium hydroxide treatment of WCS. Compared with SBM, DDGS improved dry matter intake but depressed apparent dry matter digestibility. There was trend towards improved apparent ADF digestibility with DDGS as supplemental protein. However, concentrations of ruminal metabolites (NH_3, acetate, total VFA and ratio of acetate:propionate) all indicated that under conditions of this experiment, DDGS was not supporting a ruminal environment optimum for fiber digestion. Ruminal ammonia concentrations possibly were limiting optimum microbial activity in DDGS-supplemented diets.

In the third experiment, the objectives were to characterize *in situ* ruminal disappearances of CSH, grass hay and intact WCS, and to compare the effects of treatment factors (calcium hydroxide treatment of WCS and sources of supplemental protein) on disappearance kinetics of roughages. This was run in conjunction with experiment 2 using the four fistulated cows. Extent (72 h, %), rate constants (/h), and lag times (h) of dry matter disappearance of CSH and hay were 58.3, .0398, 6.9; and 42.3, .0352, 5.0, respectively. Extent of WCS dry matter disappearance after 72-h incubation was 28.8%. A general trend in effect of calcium hydroxide treatment on disappearance kinetics suggested a depressive effect on CSH disappearance consistent with results in the second experiment. Calcium hydroxide treatment did not produce enough of the desired effect (insoluble fatty acid soap formation) to have impact on fiber digestion. There appears to be a direct negative effect of calcium hydroxide on ruminal digestion.

From results of experiments reported previously, the following conclusions may be drawn:

1. Up to 15% WCS can be added to diets of lactating cows without affecting production. At 30% inclusion, milk production and composition may be reduced.

2. Within limits of the gastro-intestinal capacity, cows were able to adjust intake by consuming more of the lower energy-density diets based on CSH. However, economics of feeding the different diets would depend on relative costs of the total diet.

3. Added limestone to provide dietary calcium at 1.29% of dry matter had no beneficial effect on milk yield and milk fat production compared with .69% calcium except when the diet was based on CSH. With CSH-based diets, the higher level of dietary calcium is recommended.

4. There is better complementarity with SBM than DDGS as supplemental proteins to WCS-containing diets. With DDGS as a source of supplemental protein, there was low ruminal ammonia concentrations perhaps imposing a limit to growth of cellulolytic ruminal organisms. If DDGS is used in WCS-containing diets, it may be beneficial to provide some form of readily available NPN in the diet.

5. Calcium hydroxide treatment of WCS was not effective as a means of alleviating depressed fiber digestibility by ruminal soaping

reactions with fatty acids. Future research should attempt to explain the reduced digestibility with DDGS supplementation.

APPENDIX

Table 38. Calculated Composition of Experimental Diets

Ration number	NE_L	TDN	CP	CF	Ca	P	Mg	K	Na	S
	(Mcal/kg)	(%)								
1	1.64	72.2	17.8	11.1	.60	.42	.32	1.34	.47	.27
2	1.51	70.0	17.9	17.0	.61	.44	.33	1.38	.50	.26
3	1.36	60.3	17.9	21.7	.60	.41	.31	1.35	.61	.28
4	1.71	74.9	17.9	13.4	.61	.44	.34	1.32	.50	.27
5	1.60	72.9	17.8	19.3	.63	.44	.30	1.40	.52	.26
6	1.42	63.0	17.9	24.0	.63	.41	.33	1.32	.64	.28
7	1.78	77.7	17.8	15.7	.63	.43	.31	1.32	.52	.26
8	1.65	75.4	17.9	21.6	.62	.44	.32	1.42	.55	.26
9	1.49	65.8	17.9	26.2	.61	.41	.31	1.33	.67	.27
10	1.62	70.9	17.8	11.1	1.21	.42	.31	1.35	.47	.27
11	1.49	68.6	17.8	17.0	1.21	.43	.33	1.38	.50	.26
12	1.33	58.9	17.7	21.6	1.22	.41	.31	1.34	.61	.28
13	1.68	73.6	17.8	13.4	1.22	.43	.34	1.31	.50	.27
14	1.55	71.3	17.7	19.2	1.24	.43	.34	1.40	.53	.26
15	1.39	61.7	17.7	23.9	1.20	.41	.33	1.31	.64	.28
16	1.75	76.3	17.5	15.7	1.24	.43	.31	1.32	.52	.26
17	1.61	74.0	17.8	21.5	1.23	.44	.32	1.43	.55	.25
18	1.46	64.4	17.8	26.2	1.22	.41	.30	1.33	.67	.27

[a] Values calculated on dry matter basis.

Table 39. Least-Squares Means for Diet Effects

									Coefficient of apparent digestibility			
Diet	DMI[a] (kg/d)	BWC[a] (kg/35 d)	Milk yield (kg/d)	Milk fat (%)	Milk protein (%)	FCM[a] (kg/d)	Fat (kg/d)	Protein (kg/d)	DM[a]	NDF[a]	ADF[a]	Cellulose
									------------	(%)	------------	
1	22.6	22.9	21.4	3.57	3.70	20.02	.76	.80	83.91	74.50	73.90	80.59
2	26.1	31.0	22.2	3.40	3.49	19.88	.73	.77	60.87	48.01	38.16	52.50
3	27.4	28.3	23.5	3.30	3.40	20.84	.76	.79	65.82	50.59	22.83	49.33
4	20.6	-2.6	22.0	3.27	3.49	19.35	.70	.76	72.28	61.50	57.59	64.21
5	19.5	10.4	21.4	3.71	3.48	20.37	.79	.73	58.97	48.56	35.42	45.72
6	25.5	45.6	23.7	3.32	3.39	21.23	.78	.80	78.06	71.68	68.64	73.27
7	20.8	33.6	23.0	3.33	3.47	20.56	.76	.79	77.76	73.16	70.31	75.64
8	20.9	-3.9	21.0	3.37	3.44	18.51	.67	.71	68.04	62.47	56.61	63.61
9	21.0	15.0	21.0	2.89	3.08	17.25	.59	.64	66.35	56.01	48.95	54.54
10	19.9	9.0	22.0	3.52	3.43	20.30	.77	.75	54.41	36.33	27.73	39.80
11	24.8	11.8	21.8	3.34	3.45	19.56	.72	.75	56.54	40.43	33.61	46.50
12	24.3	33.3	22.8	3.61	3.62	21.00	.79	.80	80.96	70.57	65.31	71.09
13	22.4	27.5	22.3	3.17	3.39	19.36	.70	.75	69.77	57.29	56.36	59.85
14	25.0	4.7	23.4	3.31	3.15	20.93	.77	.74	77.54	72.05	67.43	76.31
15	24.8	40.6	23.9	3.45	3.33	21.52	.80	.77	78.91	71.49	66.69	71.41
16	18.8	16.1	21.6	3.18	3.43	18.85	.68	.73	69.02	63.98	62.24	67.53
17	22.1	4.9	21.4	3.33	3.19	19.07	.70	.68	69.43	67.83	60.04	68.87
18	19.3	18.8	20.7	3.04	3.28	17.53	.62	.67	72.62	66.71	64.31	68.32

[a] DMI = Dry matter intake; BWC = Body weight change; FCM = 4% Fat-corrected milk; DM = Dry matter; NDF = Neutral detergent fiber; ADF = Acid detergent fiber.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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